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# THE EYE-PIGMENTARY SYSTEM OF *DROSOPHILA*

## IX. HETEROZYGOUS EFFECTS OF EYE-COLOUR GENES

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## 1. INTRODUCTION

DOMINANCE, as a basic fact of genetics, is intimately bound up with the problem of genic action in the aspects of the kinetics and thresholds of action and the quantities of end-products. Goldschmidt (1955) has fully discussed dominance as a potency effect which should be explained in terms of genic action controlling in a quantitative way some phase of the kinetics of production of active end-products. During the course of investigations on the eye-structure and pigments of a large number of eye-colour mutants of *Drosophila melanogaster* it was noted that some heterozygotes, although phenotypically normal in eye-colour, appeared to possess amounts of red and brown pigments differing from those occurring in the wild-type. It was consequently decided to institute an inquiry into the quantitative relationships of these pigments in a randomly selected series of heterozygotes. Those selected were the heterozygotes of the following mutants: vermilion (*v*), vermilion$^{36f}$ ($v^{36f}$), scarlet (*st*) and cardinal (*cd*) of the vermilion group of mutants; brown (*bw*), garnet$^{2}$ ($g^{2}$), garnet$^{3}$ ($g^{3}$), carnation (*car*) and purple (*pr*) of the ruby or garnet group; sepia (*se*) and clot (*cl*) of the dark group; apricot$^{3}$ ($w^{a3}$) of the white series of multiple alleles. These heterozygotes were obtained by crossing the named mutants with the Canton-S strain of wild-type. In the meantime, however, it was discovered that some South African strains of wild-type show significantly different amounts of eye pigments. One such strain is a high-pigment strain, called Graaff-Reinet, and obtained from the Eastern Cape Province; another strain is a low-pigment strain, called Stellenbosch, and obtained from the Western Cape Province. Heterozygotes of some of the above-named mutants were obtained by crossing with these two wild strains.

The quantitative data of pigment extracts from these heterozygotes were compared with the relative amounts of red and brown pigments, obtained for the homozygous strains in previous studies, in which the methods of quantitative estimation are fully described (Nolte, 1954, 1955, 1958).

## 2. OBSERVATIONS

In none of the heterozygotes could any deviation from the normal red colour of the wild-type eye be detected. The red and brown

pigments produced in the eyes of these heterozygotes were also normal in regard to the spectrophotometric curves obtained for the extracts

TABLE 1

*Relative amounts of red and brown pigments in the eyes of the series of mutants and their heterozygotes with the Canton-S wild-type*

| Strain | Red pigment | Brown pigment |
|---|---|---|
| + | 0·8993±0·0052 | 0·0866±0·0007 |
| *st/st* | 0·8910±0·0043 | 0·0049±0·0002 |
| *st/+* | 0·9015±0·0125 | 0·0814±0·0013 |
| *v/v* | 0·8574±0·0034 | 0·0045±0·0001 |
| *v/+* | 0·8708±0·0044 | 0·0991±0·0017 |
| $v^{36f}/v^{36f}$ | 0·8641±0·0055 | 0·0030±0·0003 |
| $v^{36f}/+$ | 0·9521±0·0021 | 0·0837±0·0019 |
| *cd/cd* | 0·9672±0·0026 | 0·0131±0·0002 |
| *cd/+* | 0·9844±0·0060 | 0·0893±0·0016 |
| *bw/bw* | 0·0110 | 0·0750±0·0006 |
| *bw/+* | 0·8791±0·0050 | 0·0824±0·0007 |
| *pr/pr* | 0·2282±0·0010 | 0·1183±0·0050 |
| *pr/+* | 0·8743±0·0112 | 0·0908±0·0011 |
| *car/car* | 0·2999±0·0044 | 0·0403±0·0011 |
| *car/+* | 0·9290±0·0095 | 0·0811±0·0015 |
| $g^3/g^3$ | 0·1900±0·0033 | 0·0409±0·0012 |
| $g^3/+$ | 0·9888±0·0125 | 0·0952±0·0011 |
| $g^2/g^2$ | 0·1384±0·0044 | 0·0274±0·0004 |
| $g^2/+$ | 0·9625±0·0125 | 0·0897±0·0004 |
| *se/se* | 0·0556±0·0006 | 0·1233±0·0013 |
| *se/+* | 0·8669±0·0042 | 0·0963±0·0018 |
| *cl/cl* | 0·3603±0·0013 | 0·1367±0·0019 |
| *cl/+* | 0·9883±0·0188 | 0·1006±0·0018 |
| $w^{a2}/w^{a2}$ | 0·0542±0·0008 | 0·0090±0·0002 |
| $w^{a2}/+$ | 0·7711±0·0103 | 0·0989±0·0038 |

TABLE 2

*Relative amounts of red and brown pigments in the eyes of some strains and their heterozygotes with the Graaff-Reinet wild-type*

| Strain | Red pigment | Brown pigment |
|---|---|---|
| + | 1·1321±0·0182 | 0·1227±0·0022 |
| Canton-S/+ | 0·9444±0·0120 | 0·1102±0·0070 |
| *v/+* | 0·9547±0·0090 | 0·1094±0·0022 |
| *cd/+* | 1·0175±0·0368 | 0·1275±0·0017 |
| *bw/+* | 0·0446±0·0156 | 0·1088±0·0035 |
| *pr/+* | 0·9065±0·0151 | 0·1331±0·0010 |
| *car/+* | 0·9269±0·0118 | 0·1033±0·0031 |
| $g^3/+$ | 1·0083±0·0216 | 0·1225±0·0022 |
| $g^2/+$ | 0·9333±0·0330 | 0·1155±0·0069 |
| *se/+* | 0·8480±0·0051 | 0·1232±0·0019 |
| *cl/+* | 1·0708±0·0041 | 0·1327±0·0021 |

in the two solvents AEA and AMA. The quantitative data for the pigments are given in tables 1-3, the measurements being of extinction ($E$) for 10 heads per 1 c.c. extract. The AEA extract was measured at 480 m$\mu$, although for the mutant sepia the peak in absorption is

at 415 mμ for the modified red pigment, and for the mutant clot it is at 430 mμ for the mixture of normal and modified red pigment (Nolte, 1954*b*). The AMA extract was measured at 444 mμ.

TABLE 3

*Relative amounts of red and brown pigments in the eyes of some strains and their heterozygotes with the Stellenbosch wild-type*

| Strain | Red pigment | Brown pigment |
|---|---|---|
| + | 0·5996±0·0106 | 0·0858±0·0012 |
| Canton-S/+ | 0·8382±0·0134 | 0·0838±0·0023 |
| *cd*/+ | 0·9312±0·0312 | 0·1063±0·0013 |
| *bw*/+ | 0·7792±0·0242 | 0·0853±0·0004 |
| *se*/+ | 0·8228±0·0098 | 0·1113±0·0028 |

## 3. DISCUSSION

As in previous investigations of this series, the norm in eye-size was used as a basis for the evaluation of quantitative pigment differences. It is evident that eye-size is difficult to measure accurately, and consequently small differences in the diameter of the eye may result in appreciable differences in pigment content as measured photometrically. None of the strains were isogenic for eye-size genes, neither for any other genes which might affect pigment quantities, and thus the standard error of the quantitative data is not always a reliable tool for estimating the significance of differences. In the case of the heterozygotes any occurrence of a pigment content higher than normal could be ascribed to an increase in eye-size as the result of heterosis. It should be noted that although heterosis is to be expected in the heterozygotes between different inbred strains, micrometric data of eye-size in all strains and their heterozygotes show no appreciable differences, and in most cases fell within the limits of the norm.

As has been established in previous studies, most eye-colour mutants have a lower content of one or the other or both of the pigments than the Canton-S wild-type. On the other hand, the mutant *cd* has a higher red pigment content, while the mutants *pr*, *se* and *cl* have a higher brown pigment content. The Graaff-Reinet strain of wild-type has a red pigment content higher than that of any other strain and a brown pigment content equal to that of *se* but less than that of *cl*. The Stellenbosch wild-type strain has a red pigment content lower than that of the Canton-S strain but an equal amount of brown pigment. The inspection and comparison of the quantitative data of tables 1-3 show a constant trend of interaction of eye-colour genes in heterozygotes : in no instance does it seem as if the dominance of the normal allele is complete for both pigments. If, however, the presence of amounts of pigment slightly in excess of those of the wild-type should be ascribed to heterosis, some of the strains should be classified as mutants of normal alleles with full dominance.

Three main types of quantitative interaction appear to occur in these data. (1) The first type is an additive effect resulting in intermediate quantities of pigment in the heterozygote, although the effect may be differential on the two pigments. Such cases in which the amounts of a pigment are more or less equal in the two strains, but with a slight increase or decrease in their heterozygote, are included in this type ; so also are those cases in which the mutant has a higher content of a pigment than the wild-type, but in the heterozygote of which the amount is intermediate. The three wild-type strains show this type of interaction in combinations, and so do the mutants *st*, *bw*, *pr* and *se* in their heterozygotes with Canton-S, as also the mutants *v*, *bw*, *car*, $g^2$, $g^3$, *se* and *cl* in their heterozygotes with Graaff-Reinet.

TABLE 4

*The dominance ratios of the normal alleles in the three wild-type strains*

| Strain | Dominance ratio of normal allele in | | | | | |
|---|---|---|---|---|---|---|
| | Canton-S | | Graaff-Reinet | | Stellenbosch | |
| | Red | Brown | Red | Brown | Red | Brown |
| *st* | 1·80 | 0·38 | ... | ... | ... | ... |
| *v* | −0·36 | 1·31 | −0·29 | 0·78 | ... | ... |
| $v^{36f}$ | 4·00 | 0·93 | ... | ... | ... | ... |
| *cd* | −1·51 | 1·08 | −0·39 | 1·09 | −0·40 | 1·57 |
| *bw* | 0·96 | 0·28 | 0·67 | 0·42 | 1·61 | 0·91 |
| *pr* | 0·93 | 0·74 | 0·50 | 5·73 | ... | ... |
| *car* | 1·10 | 0·77 | 0·51 | 0·53 | ... | ... |
| $g^3$ | 1·25 | 1·28 | 0·74 | 1·00 | ... | ... |
| $g^2$ | 1·17 | 1·10 | 0·60 | 0·85 | ... | ... |
| *se* | 0·92 | 0·47 | 0·47 | −0·67 | 1·82 | −0·36 |
| *cl* | 1·33 | 0·44 | 0·89 | −0·43 | ... | ... |
| $w^{a3}$ | 0·69 | 1·32 | ... | ... | ... | ... |

(2) The second type of interaction in the heterozygote may be called superadditive in that the amounts of both pigments are higher than in the two parental strains. In this class would fall *cd* and the garnet alleles in their heterozygotes with Canton-S. (3) In the third type of interaction there appears a quantitatively differential effect in that only one of the two pigments is present in an amount in excess of that of wild-type, while the other shows an additive effect. In this class would fall *v*, $v^{36f}$, *car*, *cl* and $w^{a3}$ in their heterozygotes with Canton-S, *cd* and *pr* in their heterozygotes with Graaff-Reinet, and *cd*, *bw* and *se* in their heterozygotes with Stellenbosch.

Another method of approach to the problem of the evaluation of the strength of dominance of eye-colour genes is to use, as a measure of average dominance the ratio : deviation of the heterozygote from the midparental value/half the parental difference. These ratios have been calculated for the normal alleles in the three wild-type strains and are given in table 4. The data of this table may be classified

under three types in regard to the strength of dominance of normal alleles. First, unity indicates full dominance of the normal allele if positive, and of the mutant gene if negative; secondly, any ratio greater than unity indicates overdominance; thirdly, any ratio smaller than unity indicates partial dominance. Some reservations must, however, be made if the significance of differences in the quantitative data of wild-type and heterozygote is taken into consideration, and if it is kept in mind that general heterosis in heterozygotes may increase body size and cause a concomitant increase in eye pigment content. Taking these factors into account, we find that in some genes a dominance ratio somewhat lower than unity may still be accepted as an indication of full dominance, and that in other cases a ratio somewhat greater than unity may result from heterosis in size, so that these genes should also be accepted as showing full dominance.

If the above-mentioned exceptions are granted as valid, we still find a series of mutant genes which exert some influence in the heterozygote. Examples are the following :—the mutants *cd* and *v* for the red pigment, and the mutants *bw*, *pr*, *car*, *se* and *cl* for the brown pigment; in addition, the mutants $v^{36f}$ and $w^{a3}$ in the Canton-S heterozygote show interaction for the red pigment, while for this pigment all the mutants show an interaction in the Graaff-Reinet heterozygotes. In general, however, it may be noted that, especially in relation to the Canton-S strain, those mutants causing large decreases in brown pigment content show heterozygous interaction for the red pigment, while mutants causing greater deviations in red pigment content show heterozygous interaction for the brown pigment. To this must be added that the first-named class is composed mainly of those mutants which, in addition, either decrease or increase the red pigment content to a slight extent, while in the second class the mutants, in addition, affect the brown pigment content, in three cases increasing the amount above that of the normal.

All these data may provide some evidence on the nature of dominance and the functions of various genes.

(1) Superior heterozygotes for monogenic differences are known in *Drosophila* (Oliver and Green, 1944). In the present investigation, however, some cases of overdominance such as occur in the heterozygotes of the garnet alleles with Canton-S wild-type, are difficult to distinguish from expressions of heterosis in size. It is doubtful whether any instance of a dominance ratio not more than 30 per cent. greater than unity can be regarded as an index of a specific interaction between the normal and mutant alleles.

(2) Different effects were observed for the two series of multiple alleles which were investigated. In the garnet alleles the relative quantities of each of the two pigments in the two homozygotes show ratios equivalent to the dominance ratios in the heterozygotes. In the vermilion alleles, however, the types of heterozygous interaction for the red pigment are of a contrasting nature : in the *v* heterozygote

the mutant allele shows slight dominance, while in the $v^{36f}$ heterozygote overdominance occurs. It may be that this difference is related to the differential functions of the alleles if the hypothesis of Green (1952) is valid, viz., $v$ and $v^{36f}$ are pseudo-alleles, and while $v$ may act during the conversion of tryptophane to an intermediate in the brown chromogen reaction chain, $v^{36f}$ may be active during the conversion of the intermediate product into formylkynurenine.

(3) The fact, which has been mentioned above, that mutants mainly affecting the brown pigment content show heterozygous interaction for the red pigment content, and that mutants mainly affecting the red pigment content show heterozygous interaction for the brown pigment content, may be related to the origin of dominance of the normal alleles of these specific genes. For these genes it may be said that the wild-type alleles show dominance in what may be regarded as the main function of the locus, *i.e.* an action in either the red or the brown pigment chains. Any secondary action of the gene, any effect on the other pigment, may then be the result of interactions between various gene-controlled processes which have arisen subsequently to the fixation of dominance. Relative dominance is a property not of the gene but of the sum of gene-controlled processes. To what extent does this concept apply in the case of the white locus which is assumed to affect both pigment chains to a more or less similar extent? Muller (1935) has demonstrated the incomplete dominance of the normal allele of white, and in the present investigation of the $w^{a3}$ allele of white there appears to be heterozygous interaction for both pigments. From the evidence of the quantitative relations between the two pigments in the series of alleles of white (Nolte, 1958) it seems that the balance between the two substrates is easily disturbed during their differentiation from a common precursor. Presumably during the evolution of dominance of the normal allele of this locus this unstable balance resulted in the fixation of dominance only in a relative way for the two pigments respectively.

(4) Some evidence may be adduced for the concept of relative dominance, or the dependence of dominance of a specific gene on the genotypic milieu. First, cases of conditioned dominance of eye-colour genes have been reported for *D. subobscura* by Crew and Lamy (1932) and for *D. melanogaster* by Schultz (1935); genes at known loci in the latter species, *e.g.* facet and purple, have been shown to be dominance modifiers of vestigial (Blanc, 1946). In such instances the presence of a specific pair of genes lowers the threshold for another pair so that the latter has some expression in the heterozygote. Secondly, the data on the heterozygotes with three different wild-type strains in the present study may be considered. These data in table 4 show some striking differences. In general, the same group of mutants (those affecting mainly the red pigment content) show interaction for the brown pigment in the heterozygotes with the Canton-S, Graaff-Reinet and Stellenbosch strains. However, these same mutants, with the addition

of the garnet alleles which show more or less full dominance of the normal allele for the red pigment in the Canton-S heterozygote, cause a more intermediate quantitative effect in the Graaff-Reinet heterozygotes. Viewed from another angle, the above-named mutants, especially *pr*, *car*, $g^3$, $g^2$, *se* and *cl*, do not show appreciable differences in relative red pigment content between their heterozygotes with Canton-S and Graaff-Reinet. On the other hand, the brown pigment content in the series of heterozygotes with Graaff-Reinet is greater than in the heterozygotes with Canton-S by amounts more or less equivalent to the difference in brown pigment content between the two wild-type strains. The mutants which affect mainly the brown pigment, for example, *v* and *cd*, show a similarity of effect in the two different heterozygotes. The mutants *se* and *cl* have normal alleles in the Canton-S strain with some degree of dominance for the brown pigment, but in the Graaff-Reinet strain the mutant alleles show a degree of dominance, also in the Stellenbosch strain for *se*.

These facts have to be reconciled with some of the theories on the origin of dominance. According to the theory of Haldane (1931) selection may have resulted in the establishment of more potent wild-type alleles, *i.e.* alleles with a considerable margin of safety. In the case of the above-mentioned series of genes the normal alleles in the Graaff-Reinet are not able to raise the amount of red pigment in the heterozygote any higher than do those of the Canton-S strain, although the Graaff-Reinet strain has appreciably more red pigment than the Canton-S ; indeed, though quantitatively inferior, Canton-S strain seems superior in the potency of its normal alleles. For these eye-colour genes there seems to be a maximum in potency which is not affected by raising the quantitative reaction very far above the threshold level. The relative dominances of these normal alleles, for this quantitative character, are dependent on the genotype. The change in the degree of dominance for the brown pigment, of the normal alleles of *se* and *cl* in Canton-S and Graaff-Reinet strains, is another example of this dependence. A third example is the quantitative differences for the brown pigment in the interactions between the group of mutants under consideration in their heterozygotes with the two wild-type strains.

The theory of the evolution of dominance by the selection of modifiers which raise the level of action in the heterozygote nearer to that in the homozygote (Fisher, 1931) could fit the facts discussed above. In this case it would be tempting to speculate on whether a modifier of a specific eye-colour gene could not be one or more of the other essential genes in the system of eye-pigment reactions, *i.e.* the whole complex of reactions is integrated through reciprocal effects of the genes on each other in the role of modifiers. If in the wild-type phenotype many degrees of normality may exist, all appearing alike on account of threshold conditions, many of the wild-type eye-pigment genes could be presumed to exist in multiple allelic forms (isoalleles).

The selection of a specific allele as a modifier of the effect of another gene could then be assumed to raise the potency of the latter in its heterozygous state—this specific allele could, in its turn, be regarded as a more potent allele in its relation to the efficiency of the whole reaction-system. Thus the selection of more potent alleles and of modifiers is interrelated, and the theories of Haldane and Fisher are integrated into a single concept.

It seems, however, as if modifying genes of another type are active in effecting the quantitative pigment differences between the wild-type strains. Since the heterozygotes of crosses between these strains possess intermediate amounts of the eye pigments, these genes may act quantitatively as systems of polygenes ; this problem is being investigated in a series of inbreeding experiments.

## 4. SUMMARY

An investigation on the quantitative relationships of the red and brown pigments in twelve eye-colour mutants of *Drosophila melanogaster* and their heterozygotes with three wild-type strains, viz. Canton-S from the United States and the South African high-pigment Graaff-Reinet and low-pigment Stellenbosch strains, have yielded the following results.

(1) In no case is there an absolute dominance of the normal alleles concurrently in the three wild-type strains. The quantitative interactions which generally appear in the heterozygotes are of three types : an additive effect for both red and brown pigments, a super-additive effect for both pigments, and a combination of the two effects, the first for one pigment and the second for the other pigment.

(2) If the average dominance of a normal allele is expressed as the ratio : deviation of the heterozygote from the midparental value/half the parental difference, more instances of nearly complete dominance become evident, but still a series of mutant genes is left with some expression in the heterozygote, generally only for one pigment in Canton-S heterozygotes but mostly for two pigments in Graaff-Reinet heterozygotes.

(3) Cases of overdominance often are difficult to distinguish from the effects of heterosis for body size in heterozygotes.

(4) Those mutants which affect mainly the red pigment show heterozygous interaction for the brown pigment, and those which affect mainly the brown pigment show heterozygous interaction for the red pigment. It is postulated that the normal alleles of these genes are dominant for the main function of the locus, but may even be recessive for secondary effects, so that dominance is relative.

(5) This relative dominance is the property of the sum of gene-controlled processes, and it is suggested that, in the case of the genes of the eye-pigmentary system, the dominance must be regarded as standing in relationship to the efficiency of the whole interaction-system. The whole complex of reactions may be integrated through

the reciprocal effects of the genes on each other as modifiers, a concept which would unite the theories of Haldane and Fisher on the evolution of dominance, that is, by the selection of more potent wild-type alleles and of modifiers respectively.

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# SOMATIC CONVERSION IN THE HEREDITY OF THE *CRUCIATA* CHARACTER IN *ŒNOTHERA*

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## 1. INTRODUCTION

In the genera *Epilobium* and *Œnothera*, types with sepaloid petals are known and these are called *cruciata* forms. The sepaloidy expresses itself in the form, colour, anatomical structure and mode of development of the petals. The character shows single factor Mendelian segregation in crosses of *E. hirsutum* var. *cruciatum* with the normal types of *E. hirsutum* and *E. parviflorum*. The normal is dominant.

In *Œnothera*, likewise, normal (*Cr*) can be clearly dominant to *cruciata* (*cr*). Usually, however, dominance is variable and the form of the corolla fluctuates between *Cr* and *cr* on different individuals or even on the same individual. This variability has a genotypic basis for the extreme flower forms commonly breed more or less true with selfing. The cause of the variability is certainly not somatic crossing-over. Mutation must therefore be taking place.

## 2. SOME EXPERIMENTS

The most exact study has been made of the cross between the following :—

(i) Normal-flowered *Œ. biennis* L. (heterogamous : *albicans* . *rubens*) ;
(ii) The strictly cruciate *Œ. atrovirens* Shull et Bartl. (heterogamous : *pingens* . *flectens*).

The hybrid *Œ.* (*atrovirens* × *biennis*) *pingens* . *rubens*, which is known as *pictirubata*, forms only pure *pingens* eggs and pure *rubens* pollen. In meiosis there are two rings : (10)+(4). Combinations of five *pingens* and two *rubens* chromosomes and vice versa might therefore be expected. They are, however, eliminated and do not appear in the progeny. Indeed they appear in the pollen as dead and empty grains additional to the active *rubens* and inactive *pingens*, which are both full grains.

$F_1$ plants with normal petals give almost all their selfed progeny normal ; *cruciata* plants give almost all *cruciata*. The results of selfing from 1949 to 1954 are shown in table 1.

For analysis of the gametes of hybrids, *cruciata* types were selected whose *cr* alleles showed themselves entirely recessive in crosses with *Cr*. Such were a small-flowered *Œ. Lamarckiana cruciata* (with flowers

no larger than *biennis*) of unknown origin and *cr sp-blandina*. Thus :—

*Cr-pictirubata* × *cr-Lamarckiana* gives

{normal (*Cr*) : *pingens* . *gaudens* (*pictilæta*)
{normal (*Cr*) : *pingens* . *velans* (*pictivelutina*).

Reciprocal cross gives

{normal (*Cr*) : *velans* . *rubens*
{[dies] *gaudens* . *rubens*.

Thus all *rubens* pollen has *Cr* and all *pingens* eggs likewise have *Cr* (instead of *cr* as expected).

Taking another experiment, the two reciprocal crosses of *Cr pictirubata* with *cr-Lamarckiana* or with *cr sp-blandina* (plate 1) show us (i) that almost all *pingens* eggs still carry *cr* and (ii) that all *rubens* pollen carries *cr* instead of *Cr*.

TABLE 1

*Progenies from* Œ. biennis × Œ. atrovirens cruciata

| Parent | $F_2$ | | $F_3$ | | $F_2+F_3$ | |
|---|---|---|---|---|---|---|
| | *Cr* | *cr* | *Cr* | *cr* | *Cr* | *cr* |
| *Cr* | 309 | 2 | 159 | 0 | 468 | 2 |
| *cr* | 15 | 243 | 4 | 145 | 19 | 388 |

The *cr-Cr* heterozygotes have therefore become homozygotes : partly *Cr-Cr* and partly *cr-cr*. Such transformation of one gene under the influence of its allele Winkler (1930) described as *conversion*. In our case it is a question of a monogenic conversion; moreover it is a somatic conversion while Winkler was considering the possibility of a reciprocal transformation occurring at meiosis.

The *Cr* homozygotes, as we saw, breed true on selfing. If the *cr* homozygotes " segregate " *Cr* plants we have to assume that it is the result, not of recombination by way of crossing-over, but of mutation or conversion. In later generations the *cr* condition can be almost as constant as the *Cr*.

If *cr-pictirubata* is back-crossed with *Cr-biennis* pollen the result is most surprising : the whole progeny is *Cr-pictirubata*. The *cr* allele in *pingens*, which showed itself as *active* in conversion, or capable of causing conversion, when it first made contact with *Cr-rubens*, is thus altered in its condition. It is weakened, shocked or " shaken " as it were, and goes under on another encounter with a fresh *Cr*.

Similarly the cross *cr-atrovirens* × *Cr-pictirubata* has mostly *cruciata* progeny. The *Cr* in *rubens* is thus also shaken by contact with *cr pingens*— although at first it has the upper hand. Corresponding results, showing the weakening of one allele by contact with the other, have come from several other combinations.

Back-crossing *Cr-pictirubata* with *cr-atrovirens* pollen we should now expect to give only *cr-pingens . flectens* (*pictiflexa*). According to its complexes *pictiflexa* is no other than *atrovirens*. The progenies of 1953 had in fact mostly sepaloid petals :

205 *cr* : 33 intermediate : 55 *Cr*.

But in the first experiment of 1926 all *pictiflexa* had been normal. So were all *pictirubata* from the cross *Cr-pictirubata* $F_1 \times$ *biennis*, v. *cruciata*. At that time, therefore, the *pingens* complex was much more inclined to be converted from *cr* to stable *Cr* than it was thirty years later. Such shifts have been repeatedly observed in the " strength " in the activity relations with the conflicting allele.

Crossing of normal *Œ. biennis* with the same small-flowered *cr-Lamarckiana* yields :

normal, *albicans . gaudens* (*albilæta*)
normal, *albicans . velans* (*albivelutina*)

If these two twin hybrids are crossed with one another reciprocally the combinations produced, *gaudens . velans* or *velans . gaudens* (which reconstitute *Lamarckiana*), are mostly *Cr*. But both complexes were originally *cr*.

Thus through living together with *Cr-albicans* the *cr* allele is in both complexes converted to *Cr*. The conversion may be complete (59 *Cr* ; 18 *Cr*) or not quite complete (113 *Cr*, 4 intermediate, 1 *cr*), or slight (38 *Cr*, 16 *cr*).

The individuals of *Cr-albilæta* and *Cr-albivelutina*, from whose crossing the last family arose, must have therefore still been partly *Cr . cr*, not yet wholly converted to *Cr . Cr*. Such wobbling in behaviour is to be found in the most various combinations. Conversion is always a potentiality. It does not show the regularity of Mendelian recombinations.

Owing to the occurrence of conversion the position of the *cr* gene in the genome is difficult to determine. Crosses of *Œ. biennis* ♂ with the homozygous taxa *Œ. Hookeri* and *Œ. Lamarckiana mut. blandina* as ♀ have indicated, however, that *cr* lies in chromosome 5·6 of *rubens*. This also contains the blunt calyx factor *sp*. Where 5·6 is missing as in *albicans* and *pingens* we can only say that *cr* is in the 5 or the 6 arm. It is not certain whether crossing-over in respect of *Cr-cr* takes place. A decision would be useful because it would indicate if the locus was far from the ends, perhaps on the borders of euchromatin and heterochromatin.

The cross of *biennis cruciata* by *Lamarckiana* normal is especially interesting because here the influence of flower size on the expression of the *cruciata* character is particularly clear. The main gene for flower size, *Co* (small) and *co* (large) lies in the 13 or 14 segment. It is so far distal that in all complex combinations *Co-co* recombine freely.

In the progeny of *cr-albilæta* $F_1 \times$ *albivelutina* $F_1$, there are three kinds of *Lamarckiana* (besides *albivelutina*) :

(i) Small-flowered individuals which have the *Co* of *albicans* in double dose. They are stable *cruciata*.

(ii) Intermediate-flowered *Co . co*. They are mostly *cruciata* but can also be normal.

(iii) Large-flowered *co . co*. They are mostly normal, sometimes with slight defects in the petals, rarely cruciate. These last, large-flowered cruciate, have almost solely *Cr*, i.e. normal offspring.

The stabilisation of the *cr* gene which has arisen from *Cr* by conversion is thus encouraged by the *Co* gene for small flowers which comes from *biennis*.

In the genomes of the homozygous *Œ. Hookeri* and *Œ. blandina* the *cr* allele behaves as though it were located in chromosome 5·6. Small-flowered and large-flowered *sp sp cr cr blandina* are fairly constant stable cruciates. The similar *sp sp cr cr Hookeri*, however, is stable when small-flowered. When large-flowered it remained cruciate only when it had received the red-nerve factor R in heterozygous state ; this came from *rubens* of *biennis* in the 1·2 chromosome which is lethal when homozygous. With homozygous *rr*, *cr* mutated to *Cr*.

Thus, it seems, the total genotype can condition the re-conversion of *cr* which has come from *Cr* (of *gaudens . velans*) back to *Cr*. And it can also condition the mutation of *cr* to *Cr* in the 5 *cr sp* 6 chromosome (of *Hookeri* and *blandina*).

## 3. DISCUSSION

The *Cr* and *cr* genes are both entirely stable in the original forms. They become labile through crossing. In one case they remain virtually unchanged ; that is in the hybrid :

*Œ.* (*biennis cruciata* × *Hookeri*) *albicans.* ʰ*Hookeri.**

In one case the dominant allele remains unchanged while the recessive becomes changed to the dominant ; that is in the twin hybrids :

*Œ.* (*biennis* × *Lamarckiana cruciata*) *albilæta* and *albivelutina*.

In this *cr-Lamarckiana* it is remarkable that the *cr* gene was very inconstant thirty-eight years ago and often gave more *Cr* than *cr* offspring. Only after a time did it become stable. And in crossing with *Cr* types it always showed itself recessive.

Apart from these exceptions the common behaviour is that both alleles become labile on crossing. And in the same $F_1$, even in the same individual, partly *Cr* is converted to *cr* and partly *cr* to *Cr*. Not only the dominant but also (in other cells) the recessive allele can be active in conversion or *prepotent* and the dominant allele can be passive in

* haplc-*Hookeri*.

*Plate*

A. (*Lamarckiana cruciata* small-flowered × *pictirubata* $F_1$ normal) *velans . rubens* normal. 1953/38.

B. (*Lamarckiana cruciata* small-flowered × *pictirubata* $F_1$ *cruciata*) *velans . rubens cruciata*. 1953/34.

C. (*Pictirubata* $F_1$ normal × *sp-blandina cruciata*) *pingens . ᵇblandina* normal. 1953/67.

D. (*Pictirubata* $F_1$ *cruciata* × *sp-blandina cruciata*) *pingens . ᵇblandina cruciata*. 1953/44.

B
D

conversion or *succumbent*. The struggle between the antagonists for prepotency lies at the genotypic level. It is often finished early in development as shown by the uniformity of many $F_1$ individuals.

The relation of valencies is of importance only when the conversion from heterozygous to homozygous condition does not take place. Prevalency or dominance belongs to the phenotypic level of activity.

What the intermediate forms of the petals mean is not clear. It is not even clear whether they are expressions of equivalence or of equipotence : (that is to say of physiological activity or of conversion activity). Mosaics of definitive *Cr* or *cr* zones can, however, arise. These show how late the conversion may occur.

Many observations suggest that the *Cr* and *cr* alleles may each be composed of a large number of units. Oehlkers has even proposed that the two may differ only in the number of their constituent units. The strongest *Cr* allele would then have the most, and the strongest *cr* the fewest, units.

Attempts to influence conversion by treatment of soaked seeds with high temperatures have not succeeded. How one allele acts on the other in conversion is quite unknown. It is not even known whether homologous segments lie together during the preparation of mitosis or whether the heterochromatin which extends on both sides of the centromere is concerned. Thus, although conversion itself can be regularly demonstrated in *Œnothera*, we still await its explanation.

It is hard to say whether conversion in *Œnothera* might happen in meiosis as well as in mitosis. But it is doubtful whether it has any connection with the irregular or partial and purely meiotic " conversion " recently discussed elsewhere, *e.g.* in *Saccharomyces* by Lindegren and Winge ; in *Neurospora* by Mitchell and Beadle ; in *Aspergillus* by Strickland ; in *Drosophila* by Goldschmidt.

## 4. SUMMARY

1. In crosses between normal and cruciate types of *Œnothera*, both of them stable, normal (*Cr*) can dominate over cruciate (*cr*).

2. Commonly the $F_1$ is variable in petal form. The normal individuals or parts of individuals then breed as *Cr Cr*, the cruciate as *cr cr*.

3. The mutational change from hetero- to homo-zygote is described as *Somatic Conversion*.

4. The dominant allele is more often active or *prepotent* in conversion than the recessive. But the recessive can also be prepotent.

5. Thus the process of conversion happens at the genotypic level. It has nothing to do with the phenotypic valency relation of dominant to recessive.

6. In the complex *rubens* of *Œ. biennis*, *cr* is linked with *sp* : *cr* thus belongs to the 5.6 chromosome, either to the 5 or the 6 arm.

7. The stability of the *cr* allele is influenced by the genotype as a

whole but the empirical observations do not reveal how somatic conversion takes place.

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# THE STABILITY OF A MULTIPLE ALLELIC SYSTEM

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## 1. INTRODUCTION

A MULTIPLE allelic system at a locus can give rise to genotypes of different viabilities. Under certain conditions the genes comprising such a system can exist in equilibrium in a large random mating population, giving rise to the situation known as a (balanced) polymorphism. Owen (1953, 1954), in discussing the problem for three alleles, has stated a set of necessary and sufficient conditions for such an equilibrium to be stable with respect to small variations of the gene frequencies about their equilibrium values. The object of this paper is to present formal proofs of these conditions for the general case of $n$ alleles, and to investigate, in some detail, the three-allele situation. In order to do this the question of the change in mean viability of the population is studied in the manner indicated by Mandel and Hughes (1958).

The situation to be investigated is that in which there are discrete, non-overlapping generations. Kimura (1956) and Penrose *et al.* (1956) have studied the problem, introducing a continuous time parameter, and replacing recurrence relations by differential equations. This procedure assumes that the change in the state of the population from one generation to the next is continuous, and is justified only in the case of infinitesimally slow selection. The analysis to be given here is free from any such restriction.

## 2. CONDITIONS FOR A NON-TRIVIAL EQUILIBRIUM

In general, consider $n$ alleles $A_1, A_2, \ldots, A_n$ at a single locus, in a large population mating at random. Let $a_{ij}$ denote the relative viability of the genotype $A_iA_j$ $(i, j = 1, 2, \ldots, n)$ ; the $a_{ij}$ thus form a symmetric matrix $(a_{ij})$ and we write

$$\Delta = \det (a_{ij}). \tag{1}$$

Let $p_i$ $(i = 1, 2, \ldots, n)$ denote the frequency of gene $A_i$ $(i = 1, 2, \ldots, n)$ in the population in any one generation, so that the $p_i$ describe the state of the population and

$$\sum_{i=1}^{n} p_i = 1, \quad 0 \leqslant p_i \leqslant 1. \tag{2}$$

Let

$$V = \sum_{i=1}^{n} \sum_{j=1}^{n} a_{ij} p_i p_j. \tag{3}$$

$V$ is thus a function of the gene frequencies, and will be called the mean viability of the population. If the population in state $p_i$ undergoes random mating, the frequencies $p_i'$ in the following generation will be given by the relations

$$Vp_i' = p_i \sum_{j=1}^{n} a_{ij}p_j \quad (\text{all } i = 1, 2, \ldots, n). \tag{4}$$

The population is in equilibrium if, for all $i$, $p_i' = p_i$. This can happen in a variety of ways in which one or more of the $p_i$ vanish. The non-trivial equilibrium, in which none of the $p_i$ is zero, is defined (if it exists) by the relations

$$\sum_{j=1}^{n} a_{ij}p_j = V, \quad \text{for all } i = 1, 2, \ldots, n. \tag{5}$$

Let $P_i$ $(i = 1, 2, \ldots, n)$ denote the values of the $p_i$ satisfying this set of inhomogeneous equations, and $V_e$ the corresponding value of $V$, then

$$P_i = \frac{\sum_{j=1}^{n} A_{ij}}{\sum_{i=1}^{n}\sum_{j=1}^{n} A_{ij}} = \frac{\sum_{j=1}^{n} A_{ij}}{D} \tag{6}$$

where $A_{ij}$ denotes the cofactor of $a_{ij}$ in $\Delta$, and writing

$$D = \sum_{i=1}^{n}\sum_{j=1}^{n} A_{ij}. \tag{7}$$

Also

$$V_e = \frac{\Delta}{D} \tag{8}$$

and setting

$$A_i = \sum_{j=1}^{n} A_{ij}, \quad \text{all } i = 1, 2, \ldots, n \tag{9}$$

(6) becomes

$$\left.\begin{aligned} P_i &= \frac{A_i}{D}, \quad \text{all } i = 1, 2, \ldots, n \\ \text{or} \qquad P_i &= \frac{V_e A_i}{\Delta}, \quad \text{all } i = 1, 2, \ldots, n. \end{aligned}\right\} \tag{10}$$

In order that an equilibrium may exist, it is necessary that the $P_i$ all be positive ; this means that the $A_i$ must all have the same sign, and this will also be the sign of $D$ and of $\Delta$. If this condition does not hold, no non-trivial equilibrium is possible. Moreover, it follows from (5) that there can be at most one non-trivial state of equilibrium.

## 3. THE CHANGE IN MEAN VIABILITY IN SUCCESSIVE GENERATIONS

In this section the variation of $V$, the mean viability, from one generation to the next will be examined when the population is in the neighbourhood of an (existent) equilibrium state ; it will be shown that the value of $V$ increases each time the population undergoes random mating.

Suppose, therefore, that a polymorphic equilibrium exists, *i.e.* the $A_i$ have the same sign for all $i = 1, 2, \ldots, n$.

Let $P_i$ $(i = 1, 2, \ldots, n)$ denote the equilibrium gene frequencies as before and $V_e$ the corresponding value of $V$.

Consider the population in the state $p_i$ $(i = 1, 2, \ldots, n)$ near the equilibrium, so that

$$p_i = P_i + x_i, \quad (i = 1, 2, \ldots, n) \tag{11}$$

where the $x_i$ are small and

$$\sum_{i=1}^{n} x_i = 0. \tag{12}$$

Also write

$$X_i = \sum_{j=1}^{n} a_{ij} x_j, \quad (i = 1, 2, \ldots, n) \tag{13}$$

and

$$\delta = \sum_{i=1}^{n} x_i X_i = \sum_{i=1}^{n} \sum_{j=1}^{n} a_{ij} x_i x_j. \tag{14}$$

In the next generation

$$\begin{aligned} Vp_i' &= p_i \sum_{j=1}^{n} a_{ij} p_j \\ &= p_i (V_e + X_i) \end{aligned} \tag{15}$$

Also

$$\begin{aligned} V &= \sum_{i=1}^{n} p_i \sum_{j=1}^{n} a_{ij} p_j \\ &= \sum_{i=1}^{n} p_i (V_e + X_i) \\ &= V_e + \sum_{i=1}^{n} x_i X_i + \sum_{i=1}^{n} P_i X_i \end{aligned}$$

*i.e.*

$$V = V_e + \delta \tag{16}$$

since

$$\begin{aligned} \sum_{i=1}^{n} P_i X_i &= \sum_{i=1}^{n} x_i \sum_{j=1}^{n} a_{ij} P_j \\ &= V_e \sum_{i=1}^{n} x_i = 0, \text{ by (12).} \end{aligned}$$

$$V' = \sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}p_i'p_j'$$

$$= \frac{1}{V^2}\sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}p_i(V_e+X_i)p_j(V_e+X_j)$$

$$= \frac{V_e^2}{V^2}\sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}p_ip_j+\frac{V_e}{V^2}\sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}p_ip_j(X_i+X_j)+\frac{1}{V^2}\sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}p_ip_jX_iX_j$$

$$= \left(\frac{V_e}{V}\right)^2 V+\frac{V_e}{V^2}\left\{\sum_{i=1}^{n} p_iX_i\sum_{j=1}^{n} a_{ij}p_j+\sum_{j=1}^{n} p_jX_j\sum_{i=1}^{n} a_{ij}p_i\right\}+\frac{1}{V^2}\sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}p_ip_jX_iX_j$$

$$= \left(\frac{V_e}{V}\right)^2 V+\frac{2V_e^2}{V^2}\sum_{i=1}^{n} p_iX_i+\frac{2V_e}{V^2}\sum_{i=1}^{n} p_iX_i^2+\frac{1}{V^2}\sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}p_ip_jX_iX_j$$

$$= \left(\frac{V_e}{V}\right)^2 (V+2\delta)+\frac{2V_e}{V^2}\sum_{i=1}^{n} p_iX_i^2+\frac{1}{V^2}\sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}p_ip_jX_iX_j \qquad (17)$$

Thus

$$V' = V+\frac{2\delta^3-3\delta^2V}{V^2}+\frac{2V_e}{V^2}\sum_{i=1}^{n} p_iX_i^2+\frac{1}{V^2}\sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}p_ip_jX_iX_j$$

so that

$$V'-V = \frac{2V_e}{V^2}\sum_{i=1}^{n} p_iX_i^2+\frac{1}{V^2}\sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}p_ip_jX_iX_j+O(x_i^3) \qquad (18)$$

where $O(x_i^3)$ denotes terms of higher degree than the second in the $x_i$.

Consider the terms

$$V_e\sum_{i=1}^{n} p_iX_i^2+\sum_{\substack{i,j=1\\ i\neq j}}^{n} a_{ij}p_ip_jX_iX_j$$

$$= \sum_{i=1}^{n} p_iX_i^2\sum_{j=1}^{n} a_{ij}P_j+\sum_{\substack{i,j=1\\ i\neq j}}^{n} a_{ij}p_ip_jX_iX_j$$

$$= \sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}P_iP_jX_i^2+\sum_{\substack{i,j=1\\ i\neq j}}^{n} a_{ij}P_iP_jX_iX_j+O(x_i^3)$$

$$= \sum_{i=1}^{n} a_{ii}P_i^2X_i^2+\sum_{\substack{i,j=1\\ i<j}}^{n} a_{ij}P_iP_j(X_i+X_j)^2+2\sum_{\substack{i,j=1\\ i<j}}^{n} a_{ij}P_iP_jX_iX_j+O(x_i^3)$$

$$= \sum_{i=1}^{n} a_{ii}P_i^2X_i^2+\sum_{\substack{i,j=1\\ i<j}}^{n} a_{ij}P_iP_j(X_i+X_j)^2+O(x_i^3)$$

Thus

$$(V'-V)V^2 = V_e\sum_{i=1}^{n} P_iX_i^2+2\sum_{i=1}^{n} a_{ii}P_i^2X_i^2+\sum_{\substack{i,j=1\\ i<j}}^{n} a_{ij}P_iP_j(X_i+X_j)^2+O(x_i^3) \qquad (19)$$

*i.e.*

$$(V'-V)V^2 = V_e\sum_{i=1}^{n} P_iX_i^2+\tfrac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij}P_iP_j(X_i+X_j)^2+O(x_i^3). \qquad (20)$$

Thus, for all sufficiently small values of the $x_i$ (that is, when the population is sufficiently near the equilibrium state) $V'-V>0$; this means that the mean viability increases from one generation to the next.

## 4. NECESSARY AND SUFFICIENT CONDITIONS FOR STABILITY OF EQUILIBRIUM

The result obtained in the last section will be used here to obtain sets of necessary and sufficient conditions for a polymorphic equilibrium to be stable.

Consider

$$V = V(p_1, p_2, \ldots, p_n) = \sum_{i=1}^{n} \sum_{j=1}^{n} a_{ij} p_i p_j \tag{21}$$

as a function of the frequencies $p_i$, which are restricted by the condition (2). Introducing the Lagrange undetermined multiplier $\lambda$, $V$ will be stationary with respect to variations in the $p_i$ subject to the restriction (2) whenever

$$\phi = \phi(p_1, p_2, \ldots, p_n) = V+\lambda \left\{ \sum_{j=1}^{n} p_j - 1 \right\} \tag{22}$$

is stationary.

This occurs whenever

$$\frac{\delta \phi}{\delta p_i} = 0, \quad \text{all } i = 1, 2, \ldots, n \tag{23}$$

which is

$$2 \sum_{j=1}^{n} a_{ij} p_j + \lambda = 0 \quad (i = 1, 2, \ldots, n) \tag{24}$$

But this set is precisely the set of equations (5) which determine the equilibrium gene frequencies $P_i$. Hence, if an equilibrium exists, it is a stationary point for $V$.

From this observation and the result obtained in the last section it is easy to see that :

(*a*) if the equilibrium corresponds to a relative maximum for $V$ then it is stable ;

(*b*) if it corresponds to a minimum value of $V$ then it is unstable ;

(*c*) if it corresponds to neither a maximum nor a minimum it is either semi-stable or neutral.

In this paper only conditions determining stability will be discussed. These may be obtained by examining the conditions which determine the nature of the stationary value of $V$. Since, by (16),

$$V = V_e + \delta \tag{16}$$

it is evident that $V$ has a relative maximum at the equilibrium if and only if the quadratic form

$$\delta = \sum_{i=1}^{n} \sum_{j=1}^{n} a_{ij} x_i x_j \tag{14}$$

is negative definite for all real $x_i$ subject to the restriction (12). A set of necessary and sufficient conditions for this to be the case is (see Ferrar (1951)) :

$$\begin{vmatrix} a_{11} & a_{1n} & 1 \\ a_{n1} & a_{nn} & 1 \\ 1 & 1 & 0 \end{vmatrix} > 0, \quad \begin{vmatrix} a_{11} & a_{12} & a_{1n} & 1 \\ a_{21} & a_{22} & a_{2n} & 1 \\ a_{n1} & a_{n2} & a_{nn} & 1 \\ 1 & 1 & 1 & 0 \end{vmatrix} < 0, \ \ldots\ldots\ldots, \tag{25}$$

$$\begin{vmatrix} a_{11} & a_{12} & \cdots & a_{1,\,n-1} & a_{1n} & 1 \\ a_{21} & a_{22} & \cdots & a_{2,\,n-1} & a_{2n} & 1 \\ \cdots\cdots & \cdots\cdots & \cdots & \cdots\cdots & \cdots\cdots & \cdots \\ a_{n-1,\,1} & a_{n-1,\,2} & \cdots & a_{n-1,\,n-1} & a_{n-1,\,n} & 1 \\ a_{n1} & a_{n2} & \cdots & a_{n,\,n-1} & a_{nn} & 1 \\ 1 & 1 & \cdots & 1 & 1 & 0 \end{vmatrix} \gtrless 0$$

according as $n$ is even or odd respectively.

These conditions, together with the conditions for an equilibrium to exist, viz. :

$$\text{the } A_i \ (i = 1, 2, \ldots, n) \text{ all have the same sign} \tag{26}$$

are necessary and sufficient for a stable equilibrium.

Introduce the notation

$$D_r = \begin{vmatrix} a_{11} & a_{12} & \cdots & a_{1r} & a_{1n} & 1 \\ a_{21} & a_{22} & \cdots & a_{2r} & a_{2n} & 1 \\ \cdots\cdots & \cdots\cdots & \cdots & \cdots\cdots & \cdots\cdots & \cdots \\ a_{r1} & a_{r2} & \cdots & a_{rr} & a_{rn} & 1 \\ a_{n1} & a_{n2} & \cdots & a_{nr} & a_{nn} & 1 \\ 1 & 1 & \cdots & 1 & 1 & 0 \end{vmatrix} \tag{27}$$

and

$$\Delta_r = \begin{vmatrix} a_{11} & a_{12} & \cdots & a_{1r} & a_{1n} \\ a_{21} & a_{22} & \cdots & a_{2r} & a_{2n} \\ \cdots\cdots & \cdots\cdots & \cdots & \cdots\cdots & \cdots\cdots \\ a_{r1} & a_{r2} & \cdots & a_{rr} & a_{rn} \\ a_{n1} & a_{n2} & \cdots & a_{nr} & a_{nn} \end{vmatrix} \tag{28}$$

Then (25) may be rewritten

$$(-1)^{r+1} D_r > 0, \quad (r = 1, 2, \ldots, n-1) \tag{29}$$

It will now be shown that an equivalent set of conditions, necessary and sufficient for a stable equilibrium, is (26) together with the set

$$(-1)^r \Delta_r > 0, \quad (r = 1, 2, \ldots, n-1) \tag{30}$$

For, let

$\alpha_{ij}^{(r)}$ $(i, j = 1, 2, \ldots, r, n)$ denote the cofactor of $a_{ij}$ in $D_r$ $(r = 1, 2, \ldots, n-1)$, and $\alpha_i^{(r)}$ $(i = 1, 2, \ldots, r, n)$ denote the cofactors of the elements of the last row and column of $D_r$ $(r = 1, 2, \ldots, n-1)$.

Then the determinant which is adjugate to $D_r$ has the shape

$$\begin{vmatrix} a_{11}^{(r)} & a_{12}^{(r)} & \dots & a_{1r}^{(r)} & a_{1n}^{(r)} & a_1^{(r)} \\ a_{21}^{(r)} & a_{22}^{(r)} & \dots & a_{2r}^{(r)} & a_{2n}^{(r)} & a_2^{(r)} \\ \dots & \dots & \dots & \dots & \dots & \dots \\ a_{r1}^{(r)} & a_{r2}^{(r)} & \dots & a_{rr}^{(r)} & a_{rn}^{(r)} & a_r^{(r)} \\ a_{n1}^{(r)} & a_{n2}^{(r)} & \dots & a_{nr}^{(r)} & a_{nn}^{(r)} & a_n^{(r)} \\ a_1^{(r)} & a_2^{(r)} & \dots & a_r^{(r)} & a_n^{(r)} & \Delta_r \end{vmatrix} \tag{31}$$

and it is clear that

$$a_{rr}^{(r)} = D_{r-1}. \tag{32}$$

Applying Jacobi's Theorem to $D_r$ and its adjugate we have

$$D_{r-1}\Delta_r - a_r^{(r)^2} = \Delta_{r-1}D_r. \tag{33}$$

Beginning with (29), $D_1 > 0$ is simply

$$2a_{1n} > a_{11} + a_{nn}$$

whence

$$4a_{1n}^2 > (a_{11} + a_{nn})^2 \geqslant 4a_{11}a_{nn}$$

*i.e.*

$$\Delta_1 < 0. \tag{34}$$

Then, applying (33) successively for $r = 2, 3, \dots, n-1$, we have $\Delta_2 > 0$, $\Delta_3 < 0$, ..., $(-1)^{n-1}\Delta_{n-1} > 0$, which is (30).

On the other hand, starting with (30), the last equation in this set is

$$(-1)^{n-1}\Delta_{n-1} > 0. \tag{35}$$

Also

$$D_{n-1} = -\sum_{i=1}^{n} A_i. \tag{36}$$

Now

$$\sum_{j=1}^{n} a_{ij}A_{jk} = \delta_{ik}\Delta_{n-1}, \quad \text{for each } i, k \tag{37}$$

where

$$\left.\begin{aligned} \delta_{ik} &= 1 \text{ if } i = k \\ &= 0 \text{ if } i \neq k \end{aligned}\right\} \tag{38}$$

Whence

$$\sum_{k=1}^{n}\sum_{j=1}^{n} a_{ij}A_{jk} = \Delta_{n-1}$$

so that

$$\sum_{j=1}^{n} a_{ij}\sum_{k=1}^{n} A_{jk} = \Delta_{n-1}$$

that is, using (9)

$$\sum_{j=1}^{n} a_{ij}A_j = \Delta_{n-1}, \quad \text{for each } i. \tag{39}$$

Since each $a_{ij}$ is positive, at least one of the $A_j$ must have the same sign as $\Delta_{n-1}$ and, by condition (26), each $A_j$ ($j = 1, 2, \dots, n$) has the

same sign as $\Delta_{n-1}$; finally, by (36), $D_{n-1}$ has the opposite sign to $\Delta_{n-1}$, whence

$$(-1)^n D_{n-1} > 0. \tag{40}$$

Successive application of (33) for $r = n-1, n-2, \ldots, 3, 2$ yields $(-1)^{n-1} D_{n-2} > 0, \ldots, D_2 < 0, D_1 > 0$ which is (29).

Thus (29) and (30), together with the equilibrium conditions (26), are two equivalent sets of conditions, necessary and sufficient for stability. Note that (30) imply (29) subject to (26), whereas the converse is true unconditionally.

In the notation of section 2

$$V_e = \frac{\Delta}{D} = -\frac{\Delta_{n-1}}{D_{n-1}}$$

since

$$\Delta_{n-1} = \Delta, \quad D_{n-1} = -D.$$

From (33) it is at once apparent that

$$D_{r-1}\Delta_r \geqslant \Delta_{r-1} D_r, \quad (r = 2, 3, \ldots, n-1)$$

so that if the conditions for stability are satisfied

$$-\frac{\Delta_r}{D_r} \geqslant -\frac{\Delta_{r-1}}{D_{r-1}} \quad (r = 2, 3, \ldots, n-1).^* \tag{41}$$

Repeated application of (41) for $r = n-1, n-2, \ldots, 3, 2$ gives

$$V_e > -\frac{\Delta_1}{D_1} = \frac{a_{1n}^2 - a_{11}a_{nn}}{2a_{1n} - a_{11} - a_{nn}}$$

But

$$a_{11}^2 + a_{1n}^2 \geqslant 2a_{11}a_{1n}$$

whence

$$a_{1n}^2 - a_{11}a_{nn} \geqslant a_{11}(2a_{1n} - a_{11} - a_{nn}) > 0.$$

Therefore

$$V_e > a_{11} \tag{42}$$

and similarly

$$V_e > a_{ii} \quad (\text{all } i = 1, 2, \ldots, n). \tag{43}$$

Thus a necessary condition for a stable equilibrium is that the viabilities of the homozygotes must be less than the mean viability of the population in the equilibrium state. This may be interpreted as a kind of generalised heterosis.

## 5. THE SPECIAL CASES OF TWO AND THREE ALLELES

In this section the case of two alleles, for which the results to be given are well known (see, *e.g.* Fisher (1922), Haldane (1926) and Wright

* The inequality is strict for $r = n-1$, since $\alpha_i^{(n-1)} = A_i \neq 0$ under the conditions for a non-trivial equilibrium; so that $\alpha_{n-1}^{(n-1)^2} = A_{n-1}^2 > 0$.

(1931)), is discussed very briefly; and that of three alleles in some detail.

### (i) *Two alleles*

Consider two alleles $A$, $B$ with genotypic viabilities as follows

| Genotype | $AA$ | $AB$ | $BB$ |
|---|---|---|---|
| Viability | $a$ | $h$ | $b$ |

The viability matrix is

$$\begin{pmatrix} a & h \\ h & b \end{pmatrix}$$

so that, in the notation of section 2,

$$\left.\begin{aligned} A_1 &= b-h \\ A_2 &= a-h \end{aligned}\right\} \tag{44}$$

and the condition (26), for an equilibrium to exist, is that these quantities must have the same sign, so that either

$$\left.\begin{aligned} &\text{(i)}\ h>a,\ h>b \\ \text{or}\ &\text{(ii)}\ h<a,\ h<b \end{aligned}\right\} \tag{45}$$

The two sets of necessary and sufficient conditions for stability are, in addition to (44), respectively

$$\begin{vmatrix} a & h & 1 \\ h & b & 1 \\ 1 & 1 & 0 \end{vmatrix} > 0 \tag{46}$$

and

$$\begin{vmatrix} a & h \\ h & b \end{vmatrix} < 0 \tag{47}$$

of which (46) is

$$(h-a)+(h-b)>0 \tag{48}$$

while (47) is

$$h^2 > ab \tag{49}$$

both of which require (45) (i).

Thus stable equilibrium between two alleles occurs if and only if the heterozygote is more viable than both homozygotes.

### (ii) *Three alleles*

Consider three alleles $A$, $B$ and $C$ at a single locus. Using the notation of Owen (1954) the genotypic viabilities are represented by the scheme

| | $A$ | $B$ | $C$ |
|---|---|---|---|
| $A$ | $a$ | $h$ | $g$ |
| $B$ | $h$ | $b$ | $f$ |
| $C$ | $g$ | $f$ | $c$ |

Let

$$\Delta = \begin{vmatrix} a & h & g \\ h & b & f \\ g & f & c \end{vmatrix} \tag{50}$$

and let the cofactor of each element in this determinant be represented by the corresponding capitals, so that

$$A = bc-f^2, \quad H = gf-hc, \text{ etc.} \tag{51}$$

Then the condition which must be satisfied for a non-trivial equilibrium to exist is that the three quantities

$$\left.\begin{matrix} A+H+G \\ H+B+F \\ G+F+C \end{matrix}\right\} \tag{52}$$

shall have the same sign.

In addition, the necessary and sufficient conditions for stability are, in the two equivalent forms, respectively

$$\begin{vmatrix} a & g & 1 \\ g & c & 1 \\ 1 & 1 & 0 \end{vmatrix} > 0, \quad \begin{vmatrix} a & h & g & 1 \\ h & b & f & 1 \\ g & f & c & 1 \\ 1 & 1 & 1 & 0 \end{vmatrix} < 0 \tag{53}$$

and

$$B<0, \ \Delta>0. \tag{54}$$

Jacobi's Theorem applied to $\Delta$ and its adjugate reveals that

$$\left.\begin{matrix} BC-F^2 = a\Delta \\ \text{and } AB-H^2 = c\Delta \end{matrix}\right\} \tag{55}$$

so that (54) imply also $A<0$, $C<0$ ; thus (54) may be stated in the symmetric form

$$A<0, \ B<0, \ C<0, \ \Delta>0. \tag{56}$$

This is the form in which the conditions are given by Owen (1954).

It is clear, also, that the same argument leads to a corresponding symmetric expression of (53).

Since

$$\begin{aligned} \Delta &= (aA+hH+gG)+(aH+hB+gF)+(aG+hF+gC) \\ &= a(A+H+G)+h(H+B+F)+g(G+F+C) \end{aligned} \tag{57}$$

it follows that, when (52) holds, the condition $\Delta>0$ is equivalent to the condition that any one (and hence all three) of $A+H+G$, $H+B+F$, $G+F+C$ be positive. Thus yet another set of conditions, necessary and sufficient for a stable equilibrium is (52) together with

$$\begin{aligned} &\text{(i) at least one of } A, B, C<0 \\ &\text{(ii) at least one of } A+H+G, H+B+F, G+F+C>0 \end{aligned} \tag{58}$$

and if these hold then in each case all three inequalities are true.

As an example of the application of these conditions, consider the case of three recessive lethals,

$$\text{i.e.} \quad a = b = c = 0. \tag{59}$$

For equilibrium it is required that the quantities

$$f(g+h-f),\ g(h+f-g) \text{ and } h(f+g-h)$$

should all have the same sign ; this requires all three quantities to be positive and $f$, $g$ and $h$ to satisfy the triangular inequalities

$$f<g+h,\ g<h+f,\ h<f+g. \tag{60}$$

Moreover (54) are then satisfied automatically so that the conditions (60) are necessary and sufficient for stability.

In the general case, it is not possible to express the stability conditions in terms of simple inequalities among the viabilities, as in the two-allele problem or the case of lethal homozygotes. The results for two alleles might suggest the analogous relations

$$a,\ b,\ c<f,\ g,\ h \tag{61}$$

(expressing the superiority of each heterozygote over each homozygote) for three alleles ; however (61) is neither necessary nor sufficient for the existence of a stable equilibrium.

For, given (61), suppose that

$$a,\ b,\ c<f,\ g<h \tag{62}$$

then

$$G+F+C = (g-a)(f-b)-(h-f)(h-g)<0$$

for all sufficiently large $h$, so that (61) is not sufficient.

On the other hand, the system

$$a = \tfrac{453}{512},\ b = 0,\ c = \tfrac{23}{32} \tag{63}$$
$$f = \tfrac{3}{2},\ g = \tfrac{151}{128},\ h = \tfrac{22}{32}$$

has a stable equilibrium with gene frequencies $(\frac{16}{29}, \frac{1}{29}, \frac{12}{29})$ and $h<a, c$ ; thus (61) is also not necessary.

However, the system (61) does imply the conditions (58), so that if an equilibrium exists under conditions (61) then it is a stable equilibrium. But, as has been shown above, (61) is not sufficient to ensure the existence of an equilibrium.

The following conditions which are necessary (but not sufficient) for a stable equilibrium will be established in succession :

(i) No heterozygote is less viable than both the associated homozygotes ; that is, we cannot have $f<b, c$ or $g<c, a$ or $h<a, b$.

(ii) Not more than one of the heterozygotes is less viable than any particular homozygote : that is, one can have, for example

$$f<a<g, h \text{ or } g<a<h, f \text{ or } h<a<f, g$$

but not

$$f, g<a \text{ or } h, f<a \text{ or } g, h<a.$$

(iii) Furthermore, not more than one heterozygote can be less viable than *any* of the homozygotes; that is, we cannot have, for example,

$$c<f<a<g<b<h.$$

(i) Follows immediately from the necessary conditions $A<0$, $B<0$, $C<0$.

(ii) Consider the general condition (43) necessary for a stable equilibrium, *i.e.*

$$V_e=\frac{\Delta}{D}>a, b, c. \tag{64}$$

This may be written

$$\frac{a(A+H+G)+h(H+B+F)+g(G+F+C)}{(A+H+G)+(H+B+F)+(G+F+C)}>a, \text{ etc.} \tag{65}$$

Hence we cannot have

$$\left.\begin{array}{l} g, h<a \\ \text{or likewise} \quad h, f<b \\ \text{or} \quad f, g<c \end{array}\right\} \tag{66}$$

Suppose now that $h<a, f<a$.

Then, by (66), $g>a$, and from condition (i) $h>b$. (67)

The following possibilities arise :

$$\begin{array}{l} (\alpha)\ f\gtrless b, f\gtrless c \\ (\beta)\ f\lessgtr b, f\gtrless c \\ (\gamma)\ f\gtrless b, f\lessgtr c. \end{array} \tag{68}$$

If either ($\alpha$) or ($\beta$) holds then

$$H+B+F=(h-a)(f-c)-(g-f)(g-h)<0. \tag{69}$$

If ($\gamma$) holds then by (66) $g>c$ and

$$\begin{aligned} H+B+F &= f(g-a)+h(g-c)+ac-g^2 \\ &< f(g-a)+a(g-c)+ac-g^2 \\ &= (g-a)(f-g)\leqslant 0. \end{aligned} \tag{70}$$

Thus in each case the relations $h<a$, $f<a$ imply $H+B+F<0$ and there is no stable equilibrium; this together with (66) establishes (ii).

(iii) We shall show that each of the inequalities

$$h<a, h<b, h<c \tag{71}$$

implies

$$f, g\gtrless a, b, c. \tag{72}$$

For, taking the first case, $h<a$ implies, using (ii), that $f, g>a$.

Suppose now that $f<b$.

Then, by (ii), $g, h > b$
and this gives rise to the two inequalities

$$\left.\begin{array}{l} a > h > b \\ b > f > a \end{array}\right\} \quad (73)$$

and

which is impossible. Thus $f > b$, and the remaining inequalities are established in the same manner.

It follows from (i), (ii), (iii) that there are only four essentially different systems which can give rise to a stable equilibrium. These may be typified as follows :

$$\left.\begin{array}{l} (a)\ a, b, c < f, g, h \\ (b)\ b < h < a, c < f, g \\ (c)\ b, c < h < a < f, g \\ (d)\ a, b < h < c < f, g \end{array}\right\} \quad (74)$$

In ($a$) all the heterozygotes are more viable than all the homozygotes ; an example of such a system has been given already (see (59), (60)).

In ($b$) one heterozygote is less viable than two homozygotes, the non-associated and either of the associated homozygotes. An example is the system (63).

In ($c$), ($d$) one heterozygote is less viable than just one homozygote ; in the former case one of the associated and in the latter the non-associated homozygote. These are exemplified respectively by the following systems :

$$a = \tfrac{5}{8}, b = 0, c = \tfrac{5}{16}$$
$$f = \tfrac{7}{4}, g = \tfrac{3}{2}, h = \tfrac{1}{2} \quad (75)$$

which has a stable equilibrium with gene frequencies $(\frac{4}{9}, \frac{1}{9}, \frac{4}{9})$ and $h < a$ ;

and

$$a = \tfrac{5}{16}, b = 0, c = \tfrac{5}{8}$$
$$f = \tfrac{7}{4}, g = \tfrac{3}{2}, h = \tfrac{1}{2} \quad (76)$$

which has a stable equilibrium with gene frequencies $(\frac{16}{128}, \frac{59}{128}, \frac{53}{128})$ and $h < c$.

## 6. SUMMARY AND CONCLUSIONS

General conditions have been obtained under which a system of $n$ alleles at a single locus can be maintained in equilibrium by natural selection in a large random mating population. It has been shown that, when the population is in the neighbourhood of such an equilibrium, the mean viability increases from each generation to the next. This establishes a criterion for the stability of the equilibrium, which yields sets of conditions which are necessary and sufficient for stability. In the general case a necessary condition for stability is that the

viability of each homozygote be less than the mean viability at equilibrium. Roughly speaking, this means that not too many heterozygotes can be less viable than any of the homozygotes. In the two-allele case this is, of course, the classic condition that the heterozygote is fitter than both homozygotes ; while in the three-allele case, it has been shown that at most one heterozygous viability may fall below that of at most two homozygotes.

The result concerning the increase in mean viability is in fact true independently of the initial state of the population. This general result depends on an interesting inequality among quadratic forms, which requires a considerably longer and more difficult proof. The special result proved here is sufficient for the present discussion.

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# SPATIAL AND FUNCTIONAL PROPERTIES OF PSEUDO-ALLELES AT THE *WHITE* LOCUS IN *DROSOPHILA MELANOGASTER*

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## 1. INTRODUCTION

THE apparent widespread occurrence of pseudoallelism has called for a re-examination of the gene concept. The need for re-examination comes primarily from seemingly conflicting results derived from independent operational procedures which describe the allelic relationships of separately occurring mutations. Thus two independent mutants are regarded as pseudoalleles if, when compounded, they produce a mutant phenotype and if single crossing-over occurs between them such that two exceptional types of gametes, one carrying neither mutant, the other both mutants, are produced. There is an added phenotypic exception concomitant to pseudoallelism: repulsion compounds of independent recessive mutants manifest a mutant phenotype, coupling compounds of the same mutants are wild-type.

On the one hand the phenotypic observation supports the conclusion that the mutants occupy identical loci, on the other hand the occurrence of crossing-over shows that they are spatially separate. The polemic incident to these observations stems from interpretations which are designed in part to reconcile the conflicts noted above. (Compare Goldschmidt, 1955; Green, 1955; Lewis, 1951, 1955; Pontecorvo, 1952, 1955.) It has been argued that the results of pseudoallelism can be interpreted best if the concept of the gene as one indivisible recombinational, functional and mutational unit is discarded. In its place is substituted a gene constituted of a number of mutational sites, functionally identical yet separable one from the other by crossing-over. This interpretation permits a formal explanation of the phenotypic and recombinational observations associated with pseudoalleles. What remains in doubt, however, is whether this concept of a large overriding functional unit within which crossing-over occurs fits all the facts and is supported by critical experimental test.

When applied to certain instances of pseudoallelism (*e.g.* the *lozenge* mutants of Drosophila or the *biotinless* mutants of Aspergillus) the concept of the overriding functional gene appears to explain all facts submitted to date. In these and similar cases of pseudoallelism all independent mutants produce either closely related or identical phenotypes, regardless of their spatial position defined by crossing-over.

Presumably since the several separable sites cannot be discriminated by any objective phenotypic criteria, it follows that the sites must be functionally identical, otherwise how could compounds produce mutant phenotypes? This may be the case for pseudoallelic arrays such as *lozenge* and *biotinless* but it is all-important to recognise that it is not universally true, and such cases of pseudoallelism as the *Star-asteroid* (Lewis, 1945) and the *bithorax-bithoraxoid* (Lewis, 1952, 1955) arrays in Drosophila provide all-important exceptions. In both instances the mutants assigned to separate sites on the basis of crossing-over can be objectively discriminated from each other on phenotypic grounds. If phenotypes are equatable to function, it follows that the pseudoalleles of the *Star-asteroid* and *bithorax-bithoraxoid* arrays are functionally as well as recombinationally distinctive. As such they do not support the concept of the overriding functional gene.

Proceeding from these considerations, the question may be asked whether it is possible to make a functional discrimination precisely paralleling spatial disposition among pseudoalleles which lack obvious phenotypic differences. The absence of non-identical phenotypes does not *a priori* mean that such spatially separate mutants are functionally identical. Many examples can be cited where non-allelic mutants produce inseparable phenotypes through alterations in different steps of biosynthetic pathways. It is therefore not inconceivable that among pseudoallelic mutants a similar situation exists and, despite the phenotypic situation, pseudoalleles govern related, but none the less separate functional steps in the production of inseparable phenotypes.

The rationale underlying the following genetic analysis may be stated as follows. In the absence of specific information on the mechanism of gene action, an indirect approach must be attempted. By subjecting individuals of different genotypes to controlled alterations in their genetic and non-genetic environments it is hoped that measurable phenotypic differences will be uncovered which permit an unambiguous separation of the mutants. If an adequate sample of independent mutants is studied, and if an exact separation can be made, such that absolute agreement between phenotypic and recombinational criteria occurs, the results then parallel the *Star-asteroid* and *bithorax-bithoraxoid* pseudoalleles and the need for the concept of separate functional and spatial genes becomes dubious.

The *white* (*w*) locus in *Drosophila melanogaster* was selected for study because it possesses a number of attributes which make it favourable for a detailed study of the functional and spatial properties of pseudoalleles. Recombination between mutants has been unequivocally demonstrated (Lewis, 1952, MacKendrick and Pontecorvo, 1952). In addition a number of different genetic factors, which alter the phenotypes of *white* pseudoalleles, have been and will be described and these are the basis for tests of function among the pseudoalleles.

## 2. MATERIALS AND METHODS

(i) *Nomenclature and origin of mutants.* A total of 36 *w* mutants is included in this report. Mutant symbols used follow the scheme listed in Bridges and Brehme (1944) with certain minor changes. For simplicity all pigmented mutants will be referred to by their superscript notation. Thus apricot ($w^a$) becomes *a*, apricot-2 ($w^{a2}$) $a^2$, cherry ($w^{ch}$), *ch*, etc. All white-eyed mutants are designated in terms of their origin. Thus spontaneous mutants are designated *s*-1, *s*-2, etc., while X-ray induced mutants are indicated by *x*-1, *x*-2, etc. The symbol *w* is reserved for the original white-eyed mutant and for referring collectively to the mutants. With but three exceptions the pigmented mutants apparently are all spontaneous in origin. The mutant *col* occurred following X-irradiation while both *dp* and *dp*2 arose following chlorethyl methane sulphate treatment.

(ii) *Design of crossing-over studies.* At the outset, it was found that crossing-over occurs between the mutants *a* and *ch*. From ♀♀ *a*/*ch* carrying in addition the closely linked marker genes *y* (yellow body, map position 0) and *spl* (split eye, 3·0) localised to the left and right of *w*, two exceptional recombination classes ($w^+$ and *a ch* coupled to same X-chromosome and white-eyed in phenotype and designated "*w*") were found. The results are included in table 2. The crossing-over data show that *a* is localised to the left of *ch*; therefore these mutants were adopted for use in testing other *w* mutants. Tester stocks carrying *a* or *ch* together with marker genes *y* and *spl* and the II and III chromosome inversions *Cy* (Curly wing) and *Ubx* (Ultrabithorax-130) balanced to *Xa* (Xasta) were constituted as follows: $y^3 su\text{-}w^a a$ *spl*; *Cy*; *Ubx*/*Xa* and *y ac ch spl*; *Cy*; *Ubx*/*Xa*. Heterozygous autosomal inversions were routinely incorporated into these tests since they strikingly increase the crossing-over frequency in the intervals studied and presumably increase recombination between pseudoalleles. The crossing-over percentages for the *y*-*w* and *w*-*spl* intervals in the absence of autosomal inversion were found to be 1·05 and 0·37 respectively, while in the presence of both *Cy* and *Ubx* the percentages increased to 4·63 and 1·87.

The linkage relation of each mutant was made by crossing mutant ♂♂ to one or the other tester ♀♀ and backcrossing $F_1$ *Cy*; *Ubx* ♀♀ to *y w spl* $sn^3$ ♂♂. Where pigmented mutants were tested both $w^+$ and double mutant recombinants, the latter without exception white-eyed and designated "*w*", were scored. Where pure white mutants were tested only the $w^+$ exceptions were sought since presumably the double mutants are white-eyed.

(iii) *Types of phenotypic modifications and their analysis.* In principle all *w* mutants were, prior to crossing-over analysis, subjected to classification based on their reactions in six different phenotypic categories. These are the following:

(1) Dosage compensation vs. non-compensation: This can be scored simply by comparing the phenotypes of ♂♂ vs. ♀♀. Non-compensation is indicated when ♀♀ are clearly darker (*i.e.* nearer wild-type) than ♂♂. In table 1, the + means compensation, the 0 non-compensation.

(2) Suppressor of $w^a$ ($su\text{-}w^a$): A recessive, sex-linked mutant tightly linked to *y* was found by Schultz to modify the phenotype of *a* flies toward wild-type. In tests reported here a spontaneous allele of the $su\text{-}w^a$ was used. Mutants were tested by crossing to $y^3 su\text{-}w^a$ flies and comparing among the $F_2$ progeny the $y^2$ and $y^+$ mutant flies. The former carry $su\text{-}w^a$. In table 1, the + indicates suppression, 0 non-suppression.

(3) Suppressor of *forked* (*su-f*): By chance it was found that the recessive sex-linked *su-f* acts as an enhancer of *a* such that flies of the genotype *a su-f* are nearly white-eyed. Mutants carrying also $f^1$ were tested for the effect of *su-f* by crossing to $f^1$ *su-f* followed by comparing among the $F_2$ progeny the phenotypes of forked and suppressed forked mutant ♂♂. In table 1 the + means enhanced by *su-f*, 0 no-enhancement.

(4) Enhancer of *eosin* (*en-w*$^e$): The origin of this sex-linked recessive modifier of *e* with map location of 32+ has been reported elsewhere (Green, 1957). The

associated plexate-like wing venation is used to identify phenotypically *en-w*$^{e}$ which is without effect on eye colour except in association with certain *w* mutants. Tests of enhancement were made by crossing mutants to *en-w*$^{e}$ followed by comparing among the $F_2$ *w* mutant ♂ progeny those with and without the enhancer. In table 1 the + signifies enhancement and o non-enhancement of the eye colour.

TABLE 1

*Summary of phenotypic interactions of* w *mutants*

| *w* mutant | Dosage compensation | *su-w*$^{a}$ | *su-f* | *su-z* | *en-w*$^{e}$ | Interaction *rb* and *g* |
|---|---|---|---|---|---|---|
| *a* | + | + | + | o | o | + |
| *a2* | + | o | o | o | o | o |
| *a3* | + | o | o | o | o | o |
| *a4* | + | o | o | o | o | + |
| *aE* | o | ... | ... | + | + | ... |
| *bf* | + | o | o | o | o | + |
| *bf*$^{2}$ | + | ... | ... | o | ... | ... |
| *bl* | + | ... | ... | o | ... | ... |
| *co* | + | o | o | o | o | + |
| *col* | + | o | o | o | o | + |
| *ch* | o | o | o | + | + | + |
| *ch*$^{2}$ | + | ... | ... | o | o | ... |
| *dp* | + | ... | ... | o | o | ... |
| $d_p$2 | + | ... | ... | o | o | ... |
| *e* | □ | o | o | + | + | + |
| *e2* | o | o | o | + | + | + |
| *ec2* | + | o | o | o | o | + |
| *M* | + | o | o | + | + | ... |
| *i* | □ | ... | ... | o | ... | ... |
| *sat* | + | o | o | o | o | + |
| *sp-w* | + | ... | ... | + | ... | ... |
| *w* | ... | o | o | + | ... | ... |
| *s-1* | ... | o | ... | o | ... | ... |
| *s-2* | ... | ... | ... | o | ... | ... |
| *s-3* | ... | ... | ... | o | ... | ... |
| *s-4* | ... | o | ... | o | ... | ... |
| *s-5* | ... | ... | ... | o | ... | ... |
| *s-9* | ... | ... | ... | o | ... | ... |
| *s-10* | ... | o | ... | o | ... | ... |
| *x-1* | ... | ... | ... | o | ... | ... |
| *x-2* | ... | ... | ... | o | ... | ... |
| *x-3* | ... | ... | ... | o | ... | ... |
| *x-4* | ... | ... | ... | o | ... | ... |
| *x-5* | ... | ... | ... | o | ... | ... |
| *x-6* | ... | o | ... | o | ... | ... |
| *x-8* | ... | ... | ... | o | ... | ... |
| *x-16* | ... | o | ... | + | ... | ... |

(5) Interaction with *ruby* (*rb*) and *garnet* (*g*) : It has been long known that certain pigmented *w* mutants interact with non-allelic eye colour mutants to produce a new phenotype lighter than the phenotype associated with either mutant (Morgan and Bridges, 1913). Thus *a rb* or *a g* flies are nearly white-eyed. Tests for interaction with *rb* and *g* were made by the usual methods utilising crossing-over to obtain the coupling combinations. In table 1 the + means the mutant interacts with *rb* and/or *g*, the o means no interaction with the phenotype inseparable from that of *rb* or *g*. A detailed study of this situation will be presented elsewhere.

(6) Suppression of *zeste* (*su-z*) : In the course of analysing the cytogenetics of the sex-linked recessive *z* mutant of *D. melanogaster*, Gans (1953) made the interesting observations that in ♀♀ homozygous for *z* and heterozygous for *w* mutants, some *w* mutants act as dominant suppressors of *z* while other *w* mutants do not.

Thus, depending on the *w* mutant, ♀♀ of the genotype *z w*$^+$/*z w* are either *zeste* or near wild-type in phenotype. Mutants were tested for suppression of *z* by obtaining ♂♂ *z w* and scoring the phenotype of their ♀ progeny after crossing to *z w*$^+$ ♀♀. The *z w* mutant ♂♂ were obtained by crossing mutant ♂♂ to *sc z ec ct* ♀♀, crossing the $F_1$ *inter se* and selecting $F_2$ ♂♂ *sc z w*. Where a pigmented *w* mutant was tested, *sc z w* ♂♂ were readily identified since *z* dilutes the mutant eye colour. No such identification could be made when a pure white-eyed mutant was used. Rather $F_2$ ♂♂ *sc z w* were collected and crossed to *z w*$^+$ ♀♀. Since *z* is located approximately equidistant between *sc* and *w*, half the *sc w* $F_2$ ♂♂ derived from ♀♀ *sc z ec ct*/*w* should be *sc z w*, half *sc z w*$^+$. Routinely 12 $F_2$ *sc w* ♂♂ were tested to assure the recovery of at least one *sc z w* ♂. In table 1 the + indicates suppression of *zeste* and the o non-suppression.

## 3. EXPERIMENTAL RESULTS

The results of the several phenotypic tests with the various *w* mutants are listed in table 1 and are for the most part self-explanatory. A few comments are, however, in order. The action of the *su-w*$^a$ and *su-f* appears to be specific for *a* and as such permits no discrimination among the *w* mutants. Of interest here are observations made with several mutants derived from *a* and tested to the *su-w*$^a$ and *su-f*. Two partial back mutations of *a* proved upon analysis to be distinctive : one is suppressed by *su-w*$^a$ and enhanced by *su-f*; the second is unchanged by either suppressor mutant. On three separate occasions *a* has mutated spontaneously to a state no longer suppressible although producing a phenotype in the absence of *su-w*$^a$ inseparable from that of *a* itself. This is not unlike the situation already described for the *forked* (*f*) locus in Drosophila where by means of X-irradiation it has been possible to mutate the suppressible *f*$^1$ mutant to an unsuppressible state (Green, 1956).

Among the tests applied, three, dosage compensation, *en-w*$^e$ and suppression of *z*, appear promising since they discriminate among several mutants. A comparison of the tests among pigmented mutants shows that with two exceptions they are in agreement. Thus all dosage compensating mutants, with the exception of *h*, are unaltered by *en-w*$^e$ and do not act as suppressors of *z*. Similarly with the exception of *i* all non-compensated mutants are enhanced by *en-w*$^e$ and function as dominant suppressors of *z*.

Within the group of white-eyed mutants discrimination can be made only in their behaviour toward *z*.

With the completion of the several phenotypic tests each mutant was tested for recombination to either the *a* or *ch* tester. Since the behaviour toward *z* provided the widest discrimination among the mutants, it was adopted as a guide for crossing-over tests. Initially all *z* suppressors were tested to *a* and all non-suppressors to *ch*.

The outcome of crossing-over tests between *z* suppressing mutants and *a* are listed in table 2. The results are self-explanatory. Wild-type exceptions arising in association with crossing-over were recovered in tests with each mutant. Among the pigmented mutants tested,

white-eyed exceptions which comprise the double mutant class were similarly recovered as reciprocals of the $w^+$ exceptions. On the basis of the distribution of the marker genes among the exceptions, each of the *z* suppressing mutants must be localised to the right of *a*. Presumed double mutants in all cases suppress *z*. The recombination relations of three other *z* suppressing mutants listed in table 1 have already been determined. Lewis (1956) has shown that *w* and *sp-w* are localised to the right of *a* and MacKendrick (1953) has localised *aE* to the right of *co* which, as will be noted below, is a putative allele of *a*.

All non-suppressors of *z* were tested for recombination with *ch* and the results are tabulated in table 3. In each instance, with the

TABLE 2

*Crossing-over between* a *and* w *mutants which suppress* z

| *w* mutant tested | Total recombinants | | Total progeny |
|---|---|---|---|
| | $y^a$ " $w$ " | $w^+$ *spl* | |
| *ch* | 1 | 2 | 32,466 |
| *e* | 3 | 1 | 21,053 |
| *e2* | 2 | 2 | 32,466 |
| *h* | 2 | 2 | 28,896 |
| *x*-16 | 0 | 2 | 30,575 |

exception of *bf*, $w^+$ individuals associated with crossing-over were found. Among pigmented mutants, including *bf*, white-eyed reciprocal exceptions were recovered as well.

That the white-eyed exceptions represent the double mutant class is demonstrated by these facts. First, the white-eyed genotype suppresses *z* and second, from ♀♀ of the presumed genotype $y^2su\text{-}w^a\ a\ ch/+ \times y\ w\ spl\ sn^3$ ♂♂, 1 ♀ $y^2su\text{-}w^a a/y\ w\ spl\ sn^3$ was recovered among 25,799 progeny. Similarly among 39,300 progeny of ♀♀ presumed to be $a^3ch\ spl/+ \times y\ w\ spl\ sn^3$ ♂, one $a^3$ ♂ was found. Thus all mutants gave exceptions which, based upon the distribution of the marker genes, show that they are, without exception, localised to the left of *ch*. In addition to the mutants of table 3, three additional non-*z* suppressing mutants, *bl*, *co* and *i* have been found by MacKendrick (1953) to be localised to the left of *ch*.

The data can be summarised as follows : among the 36 mutants studied by Lewis, by MacKendrick and here, the 8 *z*-suppressing mutants are localised to the right of *a* and the 28 non-*z* suppressing mutants are localised to the left of *ch*. There are no overlaps.

In the several instances attributed to pseudoallelism in micro-organisms the analysis of recombination among numerous independent mutants is consistent with the interpretation that the mutants are spatially separate from one another, *i.e.* they are continuously distributed along the chromosome (Benzer, 1957 ; Demerec *et al.*, 1956 ;

Pontecorvo, 1955 ; Streisinger, and Franklin, 1956). This situation finds no counterpart in Drosophila although admittedly with the exception of the *lozenge* (Green and Green, 1956) and *singed* (Hexter, 1957) pseudoalleles an insufficient number of mutants have been studied. Since phenotypic scoring in the case of *w* mutants can be made with relative ease, extensive tests were undertaken to determine whether additional loci could be uncovered among a selected group of non-*z* suppressing *w* mutants. Several mutants were tested for

TABLE 3

*Crossing-over tests between* ch *and* w *mutants all of which fail to suppress* z

| *w* mutant tested | Total recombinants | | Total progeny |
|---|---|---|---|
| | *y ac w*$^+$ | " *w* "*spl* | |
| *a2* | [illegible] | 2 | 12,840 |
| *a3* | 1 | [illegible] | 19,760 |
| *a4* | 3 | 4 | 25,440 |
| *bf* | [illegible] | 1 | 16,464 |
| *col* | 5 | 2 | 33,684 |
| *ch2* | [illegible] | [illegible] | 14,764 |
| *dp* | 2 | 0 | 35,698 |
| *dp2* | [illegible] | [illegible] | 49,670 |
| *ec2* | 4 | [illegible] | 34,310 |
| *sat* | [illegible] | [illegible] | 24,817 |
| *s-1* | [illegible] | ... | 43,245 |
| *s-2* | 1 | ... | 33,113 |
| *s-3* | 4 | ... | 20,065 |
| *s-4* | [illegible] | ... | 46,590 |
| *s-5* | 3 | ... | 54,960 |
| *s-9* | 2 | ... | 37,102 |
| *s-10* | [illegible] | ... | 30,915 |
| *x-1* | 3 | ... | 23,895 |
| *x-2* | 3 | ... | 30,830 |
| *x-3* | 5 | ... | 41,821 |
| *x-4* | [illegible] | ... | 44,100 |
| *x-5* | 7 | ... | 31,065 |
| *x-6* | [illegible] | ... | 59,905 |
| *x-8* | 6 | ... | 43,056 |

recombination with either *a*, *a2*, or *a4* or with all. Markers and inversions were included as described earlier. The results of these crosses are tabulated in table 4. Five mutants, *bf*, *col*, *sat*, *s-4 and x-6* gave *w*$^+$ recombinants when tested to either *a* or *a2*. In each case the marker associated with the *w*$^+$ exception demonstrates that spatially these mutants must all lie to the left of *a* or *a2*. From ♀♀ *bf*/*a*, white-eyed exceptions carrying markers reciprocal to those of the *w*$^+$ exception were also found. Presumably these represent the double mutant class. These data, taken together with the report of Lewis (1957) on *sp-w*, demonstrate the existence of at least four spatially discrete *w* loci.

The absence of recombinants in the extensive progeny scored from crosses *a*, *a2*, *a3*, *a4* and *co* warrant the conclusion that these mutants are truly allelic and occupy identical loci on the chromosome. In

summarising these data it should be noted that from ♀♀ *a*/*a*2 something over 400,000 progeny, equivalent to approximately $1 \cdot 6\text{-}2 \times 10^6$ progeny in the absence of autosomal inversions *Cy* and *Ubx*, were scored without detecting recombinant exceptions expected of pseudoalleles. Additional negative results were obtained with ♀♀ *a*/*a*4 where approximately 375,000 progeny, equivalent to $1 \cdot 5 \times 10^6$ without autosomal inversions, were scored. Collectively among the other crosses yielding negative results (including progeny from ♀♀ *a*/*a*3, *a*2/*a*3, *a*2/*a*4 and *a*3/*a*4)

TABLE 4

*Crossing-over among* w *mutants which fail to suppress* z

| Tester genotype | *w* mutant tested | Total recombinants | | Total progeny |
|---|---|---|---|---|
| | | $y^2w^+$ | "*w*" *spl* | |
| $y^2su\text{-}w^a a$ *spl* | *a*2 | ... | * | 226,719 |
| | *a*3 | ... | ... | 111,934 |
| | *a*4 | ... | * | 180,284 |
| | *bf* | 1 | 2 | 80,800 |
| | *co* | ... | ... | 55,275 |
| | *col* | ... | ... | 102,145 |
| | *sat* | 1 | ... | 74,840 |
| | *s*−4 | 4 | ... | 119,435 |
| | *x*−6 | 4 | ... | 113,635 |
| $y^2su\text{-}w^a a^2$ *spl* | *a*3 | ... | * | 96,939 |
| | *aRM* | ... | ... | 83,412 |
| | *aR57j* | ... | ... | 98,395 |
| | ■ | ... | ... | 89,838 |
| | *col* | ■ | ... | 79,485 |
| $y^2su\text{-}w^a a^4$ *spl* | ■ | ... | ... | 100,015 |
| | *a*3 | ... | ... | 50,210 |
| | *aRM* | ... | ... | 100,926 |
| | *aR57j* | ... | ... | 93,744 |
| | *col* | ... | ... | 51,255 |

* See text for explanation.

about 360,000 progeny, equivalent to about $1 \cdot 5 \times 10^6$ without inversions, were examined. (The partial back mutations of *a*, *aRM* and *aR57j* have been grouped with *a* in these summarisations.) It should be noted further that these negative results are not confounded by the possibility that the mutants are associated with structural changes such as a short rearrangement or loss which would preclude crossing-over. The fact that recombination occurs between *a* and four non-*z* suppressing mutants and between *a*2 and *col*, plus the fact that partial or complete back mutations of *a*, *a*2 and *a*4 have been obtained, militates for the absence of associated structural changes.

For the sake of completion it should be pointed out that while no evidence for pseudoallelism was uncovered in certain of the aforementioned crosses, two other types of exceptions were found. These

will be but briefly described here. From ♀♀ *y²su-w^a a spl/a4*, white-eyed exceptions carrying the *spl* marker have been consistently found in a frequency of about 1/10,000 progeny (17/177,784). Although incomplete, analysis of these exceptions suggests that they are possibly associated with a small loss in the *w* region presumably arising by an altered pairing of *a* and *a4* bearing chromosomes. Significantly, the reciprocal recombinant, certainly not $w^+$ in this case, has defied detection.

From ♀♀ *y²su-w^a a spl/a2*, white-eyed ♀♀ exceptions carrying the *spl* marker have been regularly recovered. Genetic and cytological analysis has shown that these ♂ lethal exceptions are associated with a loss of regions 3A4-3C1 inclusive of the salivary gland X-chromosome and are presumably the consequence of non-homologous pairing and crossing-over. Identical exceptions have been obtained from ♀♀ homozygous *a*, homozygous *a2* and *a2/a3*. The reciprocals, certainly not $w^+$, have yet to be detected. The detailed analysis of both types of exceptions is not germane to the considerations here but will be presented, in detail, elsewhere.

Finally, on three separate occasions, $w^+$ individuals have been found in the absence of crossing-over. One occurred as a single $w^+$ ; *Ubx/+* ♂ from the cross *y²su-w^a a⁴ spl/a2* ; *Cy/+* ; *Ubx/+* × *y w spl sn³*. The other two occurred as single $w^+$*/y w spl sn³* ♀♀ from crosses *y ac col spl/a2* × *y w spl sn³* and *y²su-w^a aRM spl/a2*. These exceptions are interpreted as back mutations of *a2*.

## 4. DISCUSSION

Before attempting to evaluate the results presented above, it is necessary to make clear what meaning is attached to " gene function " especially as applied to pseudoalleles. Since information on the precise mechanism of gene function is limited, any discussion of gene function must be conceptual, dealing primarily in semantics rather than specific facts.

For any given group of pseudoallelic mutants it cannot be denied that all are affecting the production of a particular phenotype in a similar manner. Specifically for the *w* pseudoalleles all mutants must be affecting eye colour synthesis similarly. What is of significance here is whether spatially discrete mutants are affecting eye colour synthesis in a similar or identical fashion. For similar functions is understood to mean that mutants are related but none the less divergent in function ; identical means the mutants have inseparable function and, in short, are duplicates of each other.

It is quite conceivable that in the case of the *w* mutants, for example, all spatial loci are functionally integrated to produce a single product—perhaps an enzyme—which is prerequisite to normal eye colour synthesis. In this sense all pseudoalleles can be said to have a single

function, the production of a specific enzyme. However, it is conceivable that each spatial locus contributes to the synthesis of the integrated product in a manner different from its neighbours. Specifically, each spatial locus may be concerned with the incorporation of a different moiety into the integrated whole and the cooperation of all loci necessary before the product can be synthesised. In this meaning—and this is the sense of usage here—each locus has a discrete function.

Any concept of the functional identity of pseudoalleles carries the tacit assumption that the spatially separable components are duplicates. A number of facts argue against such a concept. The very fact that mutations of related, recessive mutants can be found demonstrates that the mutants cannot be identical.

Almost without exception, where two pseudoalleles are coupled to the same chromosome, the resultant phenotype is equivalent to and inseparable from that of the most extreme mutant recognised in the series. As noted above, two coupled *w* mutants produce a white-eyed phenotype. Similar observations have been noted for the *lozenge* and *forked* pseudoalleles. This phenotypic effect is hardly expected under a concept of duplicate functions for those pseudoalleles, *e.g.* *w* and *f*, known to be hypomorphs.

Further, certain predictions of the genetic behaviour of pseudoalleles are possible if they are duplicates. Drawing upon experiences with such tandem duplications in Drosophila as *Bar* (Sturtevant, 1925), the *Star* duplication (Lewis, 1941), and *Beadex*-recessive (Green, 1953) it can be concluded that unequal crossing-over is a characteristic common to all. In the case of a number of arrays of pseudoallelic mutants, e.g. *white* and *lozenge*, their genetic length is comparable to that of the aforementioned duplications. Accordingly it would be predicted that unequal crossing-over would be a genetic consequence associated with pseudoalleles. Instead pseudoallelic mutants are characterised by the absence of unequal crossing-over, and one of the most consistent features of pseudoalleles seems to be the regular, two by two pairing of such loci. The absences of asymmetrical or non-two by two pairing encountered in tandem duplications can best be attributed to the fact that with pseudoalleles a sufficient degree of divergence must exist among contiguous loci to assure their regular pairing properties.

Granting the premise that the 36 independent *w* mutants studied here constitute a fair approximation of the variety of possible mutational types, a number of conclusions are warranted by the data presented. If only the crossing-over information presented here and by others is considered, it is concluded that the *w* mutants are distributed among four spatial sites. This fact, together with the negative results from extensive intralocus tests, does not support the conclusion of Pontecorvo and Roper (1956) arrived at, in part, from other considerations that " the number of mutational sites within one section of allelism (or gene) separable from one another by crossing-over may run into high

numbers". At least for Drosophila the data accumulating from studies of pseudoallelism do not support this view. Needless to say the type and nature of genic organisation peculiar to Drosophila—and presumably for other higher organisms—need not be true for micro-organisms and vice versa. In view of the relatively divergent complexities of cellular organisation and differentiation required by micro-organisms as contrasted to higher forms, it would be surprising indeed to find that they agreed in all details of their genetic organisation.

A comparison between the phenotypic interactions and crossing-over relationships of the *w* mutants shows that in two respects they are completely correlated. All eight mutants which suppress *z* have loci to the right of *a*; none of the 28 mutants which fail to suppress *z* is so located. Classification of the pigmented mutants with *en-w*$^{e}$ coincides precisely with the *z* results. All enhanced mutants occupy loci to the right of *a*; those not enhanced have loci to the left of *ch*. These facts have an all-important bearing on the question of the spatial and functional interrelationships of pseudoalleles.

The experimental data of Gans (1953) are altogether convincing that the *z* mutant depends upon the mutational and dosage conditions of the *w* locus for its phenotypic expression. The discovery that the *z* phenotype is suppressed in homozygous ♀♀ by a single dose of certain *w* mutants but not by others implies that these mutants differ in some basic functional attribute. Further, the fact that the recessive *w* mutants act as dominant suppressors suggests that this action is an inherent attribute of these mutants and is without counterpart among other *w* mutants. It is therefore not unreasonable to assume that for a particular *w* mutant the condition or substance which determines its eye phenotype is either identical with or related to that which determines its reaction toward *z*. From this assumption, it follows that in the case of two pseudoallelic mutants, each determining a white-eyed phenotype but one suppressing *z*, one not, they differ in the specific alteration producing both phenotypic properties. In short, they are functionally distinctive. Thus the *w* mutants fall into two groups; the members of each group have spatial and functional attributes which are identical and, as such, each group is an entity different from all other genes. Presumably since there are two spatial sites within each group it should be possible by means of added phenotypic tests to further divide these into discrete functional groups as well. The argument used in connection with the *z* relationship applies equally well to the *en-w*$^{e}$ behaviour and need not be repeated.

The conclusion to be drawn from these considerations is that in the case of pseudoalleles where phenotypic differences are not apparent, objective separations can be made. Thus in principle the *w* pseudoalleles are identical to *Star-asteroid* and *bithorax-bithoraxoid* in that functional and spatial groupings coincide and there is no support for the idea that pseudoalleles can best be represented by a large number of mutational sites functionally identical but separable by crossing-over.

On the basis of these considerations there is no need for two kinds of genes, those of function, those of position. Since, operationally speaking, contiguous pseudoallelic loci are separable in function as well as location they are non-allelic by the same attributes that other genes are non-allelic.

## 5. SUMMARY

1. The phenotypic and crossing-over relationships of 36 independent *white* mutants in *D. melanogaster* have been investigated.

2. On the basis of their interaction with *zeste* and *en-w*$^{e}$ the mutants can be assigned to two phenotypic groups.

3. The crossing-over studies show that (*a*) the mutants can be assigned to one of four loci and (*b*) mutants of one phenotypic group do not spatially overlap those of the second group.

4. These findings are interpreted to mean that the mutants of each group are functionally as well as spatially discrete from those of the alternative group. As such the mutants are non-allelic by available operational criteria.

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# SELECTION AND POPULATION STRUCTURE IN *LOLIUM*

## I. THE INITIAL POPULATIONS

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## 1. INTRODUCTION

In out breeding crops, such as most of the herbage grasses, the breeding system poses two related problems ; firstly, the existence of pronounced inbreeding depression, often associated with some degree of self-incompatibility, makes it difficult to base an improved strain on one individual ; and secondly, the heterozygosity of parent plants may result in the release of variation during seed multiplication. From the plant breeding point of view, it is important to know the amount of genetic variation carried within a small group of plants and its rate of release under selection, and also the minimum size of initial population necessary to maintain vigour and avoid inbreeding depression.

The aim of the present work was to study the genetic structure of certain ryegrass populations with respect to one agronomic character, the timing of inflorescence development. It is hoped that this work will assist in the effective planning of selection and breeding programmes and also serve as a pilot study for other agronomic characters in the herbage grasses.

### (i) *Choice of character*

Inflorescence development was chosen for several reasons. It is of great agronomic and adaptive importance in controlling the duration of the life-cycle and the season of flowering. A certain amount is known about its developmental basis ; most populations possess a "winter requirement" for short-day and/or low temperature before floral induction can occur, followed by a requirement for long photoperiod for floral initiation and elongation (Cooper, 1951, 1957). These developmental responses are under polygenic control and vary *between* and *within* populations (Cooper, 1954), and finally, standard field and glasshouse techniques are available for their measurement (Cooper, 1956, 1957).

### (ii) *Choice of material*

The out breeding species of *Lolium* (*L. rigidum*, *L. italicum* and *L. perenne*) are all diploid ($2n = 14$) and interfertile (Jenkin, 1954*a*, *b*, *c*). They have a geographical range in Eurasia from the Western Himalayas and the Caucasus through the Mediterranean region to North-west Europe as far north as South Norway. They have also been introduced

as forage plants or weeds to most temperate and Mediterranean climatic regions.

Three contrasting populations were chosen to cover the range of heading behaviour in the group, namely (*a*) the early-flowering winter-annual Wimmera ryegrass (*L. rigidum* Gaud.) from South Australia ; (*b*) the early-flowering hay strain of perennial ryegrass (*L. perenne* L.) Irish commercial ; (*c*) the late-flowering pasture strain of perennial ryegrass, Kent indigenous. These are all local strains which have developed under the selective action of different climates and managements. Their agronomic behaviour and past selection histories are well documented (Beddows, 1953) and will be discussed later.

### (iii) *Sequence of study*

The present paper attempts to assess the variation *between* and *within* these initial populations, as influenced by environmental and seasonal fluctuations ; and also to see how far the differences between populations in heading behaviour can be related to differences in the selective action of the climates and managements under which they have evolved. A later paper will deal with the partition of the variation within these populations into environmental and genetic components, and the further separation of the genetic variation into additive and non-additive portions, using estimates from clonal replicates and from parent-progeny regression.

The effects of selection for early and late ear emergence over three generations, starting with different population sizes exposed to different intensities of inbreeding will also be reported. The primary object was to assess the potential genetic variation carried within one, two or four individuals, and its rate of release under selection. At the same time, changes in other ear characters were studied to detect any correlated response and to measure the effect of the degree of inbreeding. These results, to be reported later, throw light on the important problem of the number of foundation plants required to maintain vigour and avoid inbreeding depression.

The present paper describes firstly, the heading behaviour of the initial populations in the field after both autumn and spring sowing, and then examines the developmental responses which underlie these field differences.

## 2. HEADING BEHAVIOUR IN THE FIELD : AUTUMN SOWING

Inflorescence development in the spring after overwintering outdoors is controlled largely by photoperiod, although the rate of inflorescence elongation is modified by spring temperature. The usual criterion recorded in the herbage grasses is the date of ear emergence, defined as that date when the tip of the third head on each plant emerges from the leaf sheath (Jenkin, 1931). The seasonal variation in ear emergence for Wimmera, Irish and Kent ryegrass is given in tables 1, 2 and 3 respectively, the years being arranged in order of

TABLE 1

*Seasonal variation in ear emergence at Aberystwyth. Wimmera ryegrass. All autumn-sown, overwintered outdoors, except where otherwise stated*

| Year | Ear emergence | | | Accum. Temp. (March-April) |
|---|---|---|---|---|
| | Mean | Var. | No. of plants | |
| 1957 | 24.0 Apr. | 42·3 | 195 | 458 |
| 1948 | 30·4 Apr. | 28·1 | 30 | 487 |
| 1949 | 3·9 May | 16·7 | 20 | 350 |
| 1956 | 6·5 May | 8·2 | 20 | 278 |
| 1958 | 7·3 May | 19·3 | 23 | 223 |
| 1955 | 10·2 May | 29·7 | 50 | 257 |
| 1947 | 13·2 May | 5·6 | 47 | 238 |
| 1949 . . | | | | |
| (Unheated house) | 19·4 Apr. | 13·8 | 20 | ... |
| (Heated house) | 16·0 Apr. | 17·3 | 20 | ... |

All one stock *ex* Waite Agricultural Institute, Adelaide.

TABLE 2

*Seasonal variation in ear emergence at Aberystwyth. Irish perennial ryegrass. All autumn-sown, overwintered outdoors*

| Year | Ear emergence | | | No. of stocks | No. of plts. | Accum. Temp. (March-April) |
|---|---|---|---|---|---|---|
| | Mean of stocks | Range of means | Mean var. | | | |
| 1957 | 28·9 Apr. | (28·1-30·3) | 22·5 | 3 | 287 | 458 |
| 1956 | 8·0 May | (6·7-9·4) | 23·0 | 4 | 184 | 278 |
| 1958 | 9·1 May | (7·5-9·4) | 20·2 | 5 | 250 | 223 |
| 1954 | 9·6 May | (8·0-10·4) | 26·9 | 8 | 234 | 257 |
| 1947 | 12·0 May | (11·7-12·3) | 11·7 | 2 | 80 | 258 |
| 1955 | 18·0 May | ... | 30·7 | 1 | 50 | 239 |

TABLE 3

*Seasonal variation in ear emergence at Aberystwyth. Kent perennial ryegrass. All autumn-sown, overwintered outdoors*

| Year | Ear emergence | | | No. of stocks | No. of plts. | Accum. Temp. (April-May) |
|---|---|---|---|---|---|---|
| | Mean of stocks | Range of means | Mean var. | | | |
| 1957 | 22·2 May | (20·8-23·2) | 44·6 | 3 | 292 | 495 |
| 1956 | 24·4 May | (22·5-27·1) | 26·5 | 6 | 268 | 450 |
| 1954 | 24·4 May | ... | 26·4 | 1 | 125 | 464 |
| 1958 | 29·0 May | (25·6-31·0) | 21·3 | 5 | 176 | 460 |
| 1955 | 1·7 June | ... | 24·5 | 1 | 50 | 446 |

earliness. All the material had been sown in the previous summer and overwintered as spaced plants in the field. These tables also include the accumulated temperatures above 42° F. for March and April for Wimmera and Irish, and for April and May for Kent, that is approximately for the two months prior to ear emergence.

The heading and temperature data up to and including 1950 refer to Penglais, Aberystwyth, 250 ft. above sea-level on a west-facing slope overlooking the sea. From 1954 onwards the data are taken from Gogerddan, Aberystwyth, 120 ft. above sea-level, but in an inland valley 3 miles from the sea, and liable to temperature inversions at night (Howe, 1953).

In Wimmera ryegrass, the date of ear emergence is comparatively early, but appreciable year-to-year variation occurs, from 24th April in a warm spring (1957) to 13th May in a cold spring (1947). Table 1 also gives the heading behaviour of Wimmera seedlings sown in the autumn of 1948 and overwintered in heated and unheated greenhouses. The higher temperatures in the greenhouses have accelerated ear emergence, in the heated house, by 18 days. As all the Wimmera seed came from the same source (the Waite Agricultural Institute, Adelaide), no comparison of different stocks is possible.

In Irish ryegrass, the date of ear emergence is again comparatively early, varying from 28th April in a warm spring (1957) to 18th May in a cold spring (1955). In Kent ryegrass, by contrast, ear emergence is late, ranging from 22nd May in 1957 to 2nd June in 1955. The year-to-year variation is not, however, as great as in Wimmera or Irish. The samples of Irish and Kent ryegrass tested represent ordinary commercial stocks, and in tables 2 and 3 the results from different stocks in the same year are bulked. Table 4, however, gives a comparison of three stocks of Irish and three of Kent for one early year (1957) and one late year (1958). In both Irish and Kent, differences between stocks are very small compared to the difference between the two strains. These findings are supported by the more detailed records of Beddows (unpublished) on the seasonal variation in ear emergence of a wide range of species and strains of herbage grasses.

Estimates of the error variance of single spaced plants derived from clonal replicates of Irish and Kent ryegrass in 1955 to 1958 are given in table 5. No significant differences were detected between blocks in the same field in any of these years and it is evident that, within both strains, the greater part of the variation is genetic.

Inspection of tables 1, 2 and 3 indicates that for Wimmera and Irish ryegrass and to a lesser degree for Kent, there is a close relation between spring temperature and the earliness of ear emergence. Previous transplant studies (Cooper, 1952) using clonal material of early-, medium-, and late-flowering perennial ryegrass showed a good correlation between the date of ear emergence within each group and the accumulated temperature above 42° F. during inflorescence development. In the present work, therefore, the regressions of date of

ear emergence on accumulated temperature for a number of years were calculated, using March and April temperatures for Wimmera and Irish, and April and May temperatures for Kent ryegrass (fig. 1).

TABLE 4

*Variation between stocks in ear emergence. Irish and Kent perennial ryegrass. All autumn-sown, overwintered outdoors*

| Strain | Stock | Ear emergence | |
|---|---|---|---|
| | | 1957 | 1958 |
| Irish . . . | Ba 6650 | 30·3 Apr. | 9·4 May |
| | Ba 6738 | 28·4 Apr. | 8·1 May |
| | Ba 6773 | 28·1 Apr. | 7·5 May |
| | Mean | 28·9 Apr. | 8·3 May |
| Kent . . . | Ba 6565 | 20·8 May | 25·6 May |
| | Ba 6622 | 22·8 May | 29·3 May |
| | Ba 6740 | 23·2 May | 29·7 May |
| | Mean | 22·3 May | 28·2 May |

Differences between stocks, non-significant in Kent :
significant at $P = 0{\cdot}05$-$0{\cdot}01$ in Irish.

In Irish and Wimmera, the regressions are highly significant and more than 60 per cent. and 70 per cent. respectively of the year-to-year variation can be attributed to accumulated temperatures. In Kent,

TABLE 5

*Error variation in ear emergence from replicated clones. Irish and Kent ryegrass*

| Year | Error variance of single plant | | Error variance of mean of 50 plants | |
|---|---|---|---|---|
| | Irish | Kent | Irish | Kent |
| 1955 | 2·75 | 1·25 | 0·055 | 0·025 |
| 1956 | 2·86 | 3·02 | 0·057 | 0·060 |
| 1957 | 4·25 | 2·93 | 0·085 | 0·059 |
| 1958 | 2·86 | 2·81 | 0·057 | 0·056 |

where there is less year-to-year variation, the regression is barely significant, accounting for less than 20 per cent. of the seasonal variation. In this strain, by the time the photoperiodic threshold for floral initiation has been reached, spring temperature is probably no longer limiting.

From these results, one may conclude that in overwintered material of Wimmera, Irish and Kent ryegrass, all plants within each strain head fairly uniformly, Wimmera and Irish at the beginning of May

and Kent in late May. Considerable year-to-year variations occur, these being attributable largely to differences in spring temperature, once the critical photoperiod has been reached. In all strains there is a tendency for a warm spring both to accelerate heading and also to increase the variance. The greater part of the variation within samples is genetic ; slight differences between stocks may occur in both Irish and Kent.

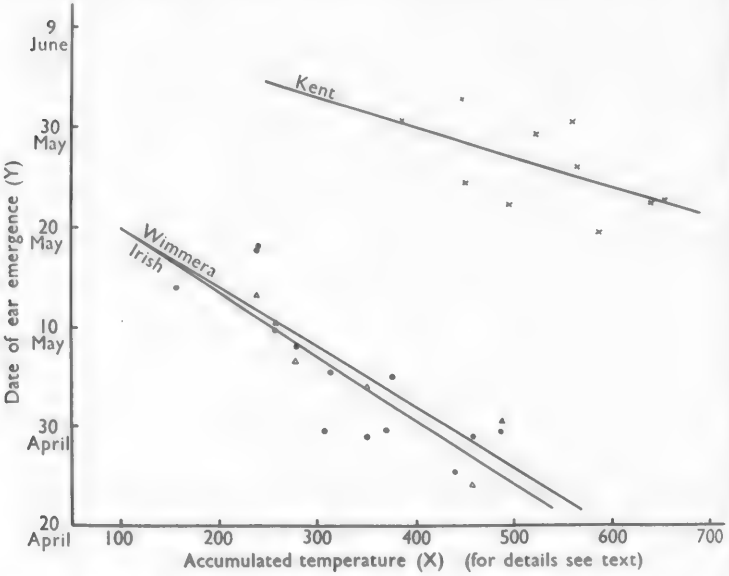


FIG. 1.—Effect of spring temperature on inflorescence development in Wimmera, Irish and Kent ryegrass.

Wimmera . $Y = 25{\cdot}0 - 0{\cdot}059\ (\pm 0{\cdot}016)X$
Irish . . $Y = 26{\cdot}4 - 0{\cdot}064\ (\pm 0{\cdot}015)X$
Kent . . $Y = 42{\cdot}3 - 0{\cdot}031\ (\pm 0{\cdot}015)X$

(Y = date in May, and X = accumulated temperature.)

## 3. HEADING BEHAVIOUR IN THE FIELD : SPRING SOWING

When seedlings are overwintered outdoors, all the plants produce heads the following spring, and the range in ear emergence within each strain is comparatively small. The date of ear emergence is, in fact, a valuable agronomic character for separating strains into different maturity groups (Hawkins, 1950 ; Thomson and Seaton, 1956).

When sowing is delayed until the spring, the picture is rather different, as can be seen from tables 6 to 8 which show the effects of spring sowing on Wimmera, Irish and Kent respectively.

In Wimmera, all seedlings produce heads even when sown as late as mid-April, but sowing after the end of January leads to an increasing

TABLE 6

*Effect of date of sowing on ear emergence at Aberystwyth. Wimmera ryegrass*

| Year of heading | Sowing date | Ear emergence | | |
|---|---|---|---|---|
| | | Mean | Var. | No. of plts. |
| 1947 | 15 June 46 | 13·2 May | 5·64 | 47 |
| | 11 Feb. 47 | 22·0 May | 141·0 | 30 |
| | 15 Apr. 47 | 10·8 July | 447·2 | 29 |
| 1949 | 15 Oct. 48 | 3·9 May | 16·7 | 20 |
| | 29 Jan. 49 | 17·4 May | 53·9 | 20 |
| | 29 Mar. 49 | 3·3 July | 241·1 | 20 |
| 1955 | 23 Aug. 54 | 10·2 May | 29·7 | 50 |
| | 7 Jan. 55 | 21·0 May | 28·5 | 46 |
| | 3 Mar. 55 | 25·6 June | 477·0 | 49 |
| 1957 | 8 Aug. 56 | 24·3 Apr. | 31·6 | 95 |
| | 30 Jan. 57 | 25·2 May | 196·0 | 41 |

All seedlings head in all sowing dates.

TABLE 7

*Effect of date of sowing on ear emergence at Aberystwyth. Irish perennial ryegrass*

| Year of heading | Sowing date | Percentage heading | Ear emergence | | |
|---|---|---|---|---|---|
| | | | Mean (or median) | Var. | No. of plts. |
| 1947 | 15 June 46 | 100·0 | 14·4 May | 8·6 | 89 |
| | 2 Nov. 46 | 100·0 | 11·8 May | 10·4 | 40 |
| | 20 Jan. 47 | 95·0 | (1 June) | ... | 40 |
| | 11 Feb. 47 | 66·7 | (28 July) | ... | 60 |
| | 21 Feb. 47 | 67·5 | (8 Aug.) | ... | 40 |
| | 20 Mar. 47 | 32·5 | ... | ... | 40 |
| | 1 Apr. 47 | 10·0 | ... | ... | 120 |
| 1949 | 15 Oct. 48 | 100·0 | 12·3 May | 13·0 | 20 |
| | 29 Jan. 49 | 80·0 | (8 July) | ... | 20 |
| | 29 Mar. 49 | 10·0 | ... | ... | 20 |
| | 25 Apr. 49 | nil | ... | ... | 60 |
| 1955 | 23 Aug. 54 | 100·0 | 17·9 May | 30·7 | 50 |
| | 7 Jan. 55 | 100·0 | 2·4 June | 16·2 | 49 |
| | 3 Mar. 55 | 45·4 | ... | ... | 44 |
| | 27 Apr. 55 | nil | ... | ... | 120 |
| 1956 | 12 Aug. 55 | 100·0 | 8·7 May | 12·4 | 43 |
| | 5 Jan. 56 | 93·3 | (7 June) | ... | 90 |
| | 13 Mar. 56 | 40·0 | ... | ... | 90 |
| | 8 May 56 | 1·1 | ... | ... | 90 |
| | 6 July 56 | nil | ... | ... | 90 |
| 1957 | 13 Aug. 56 | 100·0 | 28·1 Apr. | 25·6 | 100 |
| | 30 Jan. 57 | 58·8 | (30 June) | ... | 34 |

The same stock was used within any one year.

delay in the mean ear emergence date (up to mid-July following an April sowing) and also to a greatly increased range and variance.

In Irish and Kent ryegrass, a progressive delay in sowing after January not only leads to a delay in ear emergence, but also to a regular decrease in the proportion of plants which head (fig. 2). In

TABLE 8

*Effect of date of sowing on ear emergence at Aberystwyth. Kent perennial ryegrass*

| Year of heading | Sowing date | Percentage heading | Ear emergence | | |
|---|---|---|---|---|---|
| | | | Mean (or median) | Var. | No. of plts. |
| 1947 | 15 June 46 | 100·0 | 29·4 May | 24·0 | 30 |
| | 11 Feb. 47 | 15·0 | ... | ... | 60 |
| | 1 Apr. 47 | nil | ... | ... | 30 |
| | 17 Apr. 47 | nil | ... | ... | 30 |
| 1949 | 15 Oct. 48 | 100·0 | 28·7 May | 18·2 | 20 |
| | 29 Jan. 49 | 50·0 | 21 Aug. | ... | 20 |
| | 29 Mar. 49 | nil | ... | ... | 20 |
| | 25 Apr. 49 | nil | ... | ... | 30 |
| 1955 | 23 Aug. 54 | 100·0 | 1·7 June | 24·5 | 50 |
| | 7 Jan. 55 | 96·0 | (17 June) | ... | 50 |
| | 3 Mar. 55 | 12·2 | ... | ... | 49 |
| | 27 Apr. 55 | nil | ... | ... | 50 |
| 1956 | 12 Aug. 55 | 100·0 | 24·3 May | 31·0 | 40 |
| | 8 May 56 | nil | ... | ... | 90 |
| 1957 | 13 Aug. 56 | 100·0 | 22·8 May | 40·0 | 94 |
| | 30 Jan. 57 | 31·6 | ... | ... | 38 |

The same stock was used within any one year.

Irish ryegrass very few plants produce heads when sown after the middle of April, while in Kent the critical date is earlier, about the end of March. For any particular sowing date from January to April, the proportion of plants which form heads is higher in Irish than in Kent. Although fig. 2 is constructed from the results of several years, the decrease in heading with advancing sowing date is very regular, suggesting that the degree of winter induction provided by a particular sowing date is fairly uniform from year to year.

Within each strain, however, there is a range in the rapidity with which floral induction occurs after a spring sowing. A more detailed picture of this variation is given in fig. 3 which shows the distribution of ear emergence in Irish ryegrass after a series of sowings from autumn 1946 to summer 1947. After an autumn sowing all plants headed fairly uniformly as shown by the steep and linear probit line ; as the

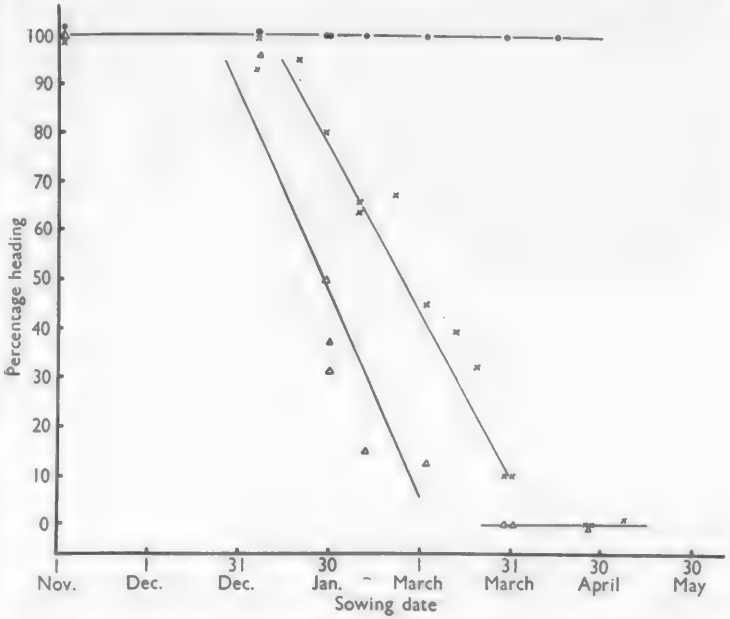


FIG. 2.—Effect of sowing date on inflorescence development in Wimmera (●), Irish (✕), and Kent (△) ryegrass.

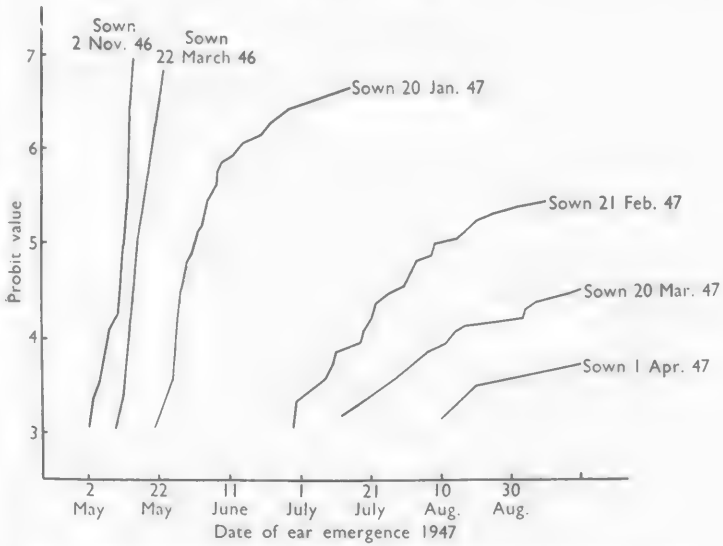


FIG. 3.—Effect of sowing date on inflorescence development in Irish perennial ryegrass. X 2

sowing date becomes later an increasing number of plants show a delay in heading and eventually fail to head at all. Spring sowing therefore reveals differences between genotypes in " winter requirement " for short-day and/or low temperature before floral induction. At the one extreme there are plants which fail to become induced when sown at the end of January, while at the other end of the range, some will head when sown in mid-April. The genetic control of these differences in inductive requirement will be considered in a later paper.

These results suggest that in the field material, while date of heading after overwintering is a measure of response to photoperiod modified in its expression by spring temperature, the extent of heading after a spring sowing is a measure of the winter requirement for short-day and/or low temperature. For both responses considerable differences between and within strains can be detected.

## 4. DEVELOPMENTAL RESPONSES : FLORAL INDUCTION

While these field sowings can distinguish between strains and also detect variation within strains, laboratory and greenhouse tests provide more rapid and reproducible results and also give information on the developmental responses to temperature and photoperiod which underlie the differences in field behaviour. The three initial strains, Wimmera, Irish and Kent have therefore been tested for the flowering responses under controlled conditions.

Earlier studies (Cooper, 1957) had shown that Wimmera ryegrass had no obligatory requirement for low temperature before floral induction, although in many plants heading could be accelerated by cold treatment. In Irish and Kent ryegrass, on the other hand, most plants had an obligatory requirement for low temperature, this being greater for Kent than for Irish. In all strains, considerable genetic variation in response to low temperature occurred within the strains. Later work, however, has suggested that short-day can also bring about floral induction, so these greenhouse tests were designed to include both short-day and low temperature treatments.

### (i) *Response to induction* : *August* 1956

The following inductive treatments were given to Wimmera, Irish and Kent ryegrass :

(*a*) low temperature alone (0-4° C.) given to the germinating seeds (for details see Cooper, 1956) ;

(*b*) short-day alone (8 hr. photoperiod) given to the young seedlings in a warm greenhouse.

Each treatment was given for 4 and 8 weeks, and after induction all material was transferred, on 1st August 1956, to continuous light in a warm greenhouse. The untreated controls were sown to germinate on the date of transfer.

Leaf number on the main shoot before heading was recorded for each seedling, while for the short-day treatments the leaf number at transfer to continuous light was also recorded, and the leaf number between transfer and heading calculated as the measure of reproductive development. Seedlings which had not headed by the 20th leaf stage were recorded as non-heading.

The results are given in table 9. Wimmera ryegrass showed no obligatory requirement for either short-day or low temperature but in the controls the leaf numbers ranged from 6 to 18 with a mean of 11·2 and a

TABLE 9

*Response to low temperature and short-day induction. All transferred to continuous light 1st August 1956*

| Strain | Exposure (wks.) | Low temperature | | | Short-day (8 hr.) | | |
|---|---|---|---|---|---|---|---|
| | | Percentage heading | Leaf no. | | Percentage heading | Leaf no. | |
| | | | Mean | Var. | | Mean | Var.* |
| Wimmera . | 0 | 100·0 | 11·2 | 6·4 | 100·0 | 11·2 | 6·4 |
| | 4 | 100·0 | 5·5 | 1·6 | 100·0 | 8·0 | 8·1 |
| | 8 | 100·0 | 4·3 | 0·2 | 100·0 | 4·1 | 1·2 |
| Irish . . | 0 | 11·8 | ... | ... | 11·8 | ... | ... |
| | 4 | 19·2 | ... | ... | 25·6 | ... | ... |
| | 8 | 81·3 | (7·9) | ... | 27·0 | ... | ... |
| Kent . | 0 | nil | ... | ... | nil | ... | ... |
| | 4 | 4·0 | ... | ... | 16·0 | ... | ... |
| | 8 | 25·7 | ... | ... | 30·0 | ... | ... |

* Leaf no. after transfer. Each sample based on 80 initial seedlings.
For leaf numbers, significant difference at $P = 0{\cdot}05$ is 0·72.
For percentage heading, significant difference at $P = 0{\cdot}05$ varies from 5·0 per cent. at the 0 and 100 per cent. levels to 22·5 per cent. at the 50 per cent. level.

variance of 6·45. Four weeks' low temperature resulted in complete induction in nearly all seedlings and after 8 weeks' treatment the leaf number was reduced to 4 or 5 in all plants. Four weeks of short-day also had an inductive effect, though not as great as the same period of cold, and 8 weeks' short-day was enough for complete induction.

In Irish ryegrass, 11·8 per cent. of the seedlings headed without any inductive treatment, but even 8 weeks of cold induced heading in only 81·3 per cent. of the plants. Again the inductive effect of short-day was not as great as the same period of low temperature.

In Kent, on the other hand, no plants produced heads without short-day or low temperature induction, but the effect of short-day was equal to that of cold. Kent evidently has a greater requirement for induction than Irish, and only 25 per cent. to 30 per cent. of the plants headed after 8 weeks' treatment in this experiment.

These results, while emphasising the difference between the annual Wimmera and the two perennial strains, also reveal differences within

strains in response to induction. In Irish, for instance, a few plants are able to head without either short-day or cold, while others require more than 8 weeks' exposure.

### (ii) *Response to induction : January* 1958

This first experiment did not provide complete induction in the perennial strains and the test was therefore repeated using longer

TABLE 10

*Response to low temperature and short-day induction. All transferred to continuous light on 30th January 1958*

| Strain | Exposure (wks.) | LT alone | | | LT+SD | | | SD alone | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Per cent. heading | Leaf no. | | Per cent. heading | Leaf no. | | Per cent. heading | Leaf no.* | |
| | | | Mean | Var. | | Mean | Var. | | Mean | Var. |
| Wimmera | 0 | 100·0 | 10·7 | 17·97 | 100·0 | 10·7 | 17·97 | 100·0 | 10·7 | 17·97 |
| | 4 | 100·0 | 4·8 | 0·48 | 100·0 | 5·4 | 1·31 | 100·0 | 5·1 | 0·54 |
| | 8 | 100·0 | 5·0 | 0·45 | 100·0 | 4·8 | 0·25 | 100·0 | 4·6 | 0·39 |
| | 12 | 100·0 | 5·0 | 0·50 | 100·0 | 4·3 | 0·28 | 100·0 | 4·6 | 0·69 |
| Irish | 0 | 42·5 | ... | ... | 42·5 | ... | ... | 42·5 | ... | ... |
| | 4 | 57·5 | (16·3) | ... | 48·7 | ... | ... | 90·0 | (7·7) | ... |
| | 8 | 94·7 | (10·0) | ... | 97·5 | (8·0) | ... | 100·0 | 4·7 | 0·57 |
| | 12 | 100·0 | 10·0 | 5·09 | 100·0 | 8·5 | 3·13 | 100·0 | 4·2 | 1·45 |
| Kent | 0 | 7·5 | ... | ... | 7·5 | ... | ... | 7·5 | ... | ... |
| | 4 | 48·4 | ... | ... | 12·5 | ... | ... | 84·2 | (9·4) | ... |
| | 8 | 82·2 | (11·4) | ... | 94·7 | (9·2) | ... | 100·0 | 5·3 | 1·42 |
| | 12 | 100·0 | 11·6 | 5·73 | 100·0 | 9·3 | 2·65 | 100·0 | 5·1 | 1·34 |

* Leaf no. after transfer. Each sample based on 40 initial seedlings.
For leaf no., significant difference at $P = 0{\cdot}05$ is 0·96.
For percentage heading, significant difference at $P = 0{\cdot}05$ varies from 18 per cent. at the 0 and 100 per cent. levels to 26 per cent. at the 50 per cent level.

inductive periods, and also applying short-day and low temperature concurrently. The following treatments were given to Wimmera, Irish and Kent ryegrasses : (*a*) low temperature alone (0-4° C.) ; (*b*) short-day alone (8 hr. photoperiod) and (*c*) short-day and low temperature concurrently. In the last case, the seedlings were grown in vermiculite until the first leaf emerged, then transferred to the refrigerator and given 8 hr. photoperiod, using four 15-watt " daylight " fluorescent tubes (intensity at seedling level, 90 ft. candles).

Each treatment was given for 4, 8 and 12 weeks, and the sowings arranged so that all material was transferred to continuous light in a warm greenhouse on the same date, 30th January 1958. The untreated controls were sown to germinate on this date. Records of leaf number before heading were taken as in the previous experiment.

The results of these induction treatments (table 10) confirmed the

earlier findings and also showed that low temperature plus short-day had slightly more effect than low temperature alone. Wimmera again proved to have no obligatory requirement for short-day or cold, but the controls showed a range of leaf numbers from 5 to 23 with a mean of 10·7. Four weeks of low temperature alone, or of short-day alone, or of both, were sufficient for complete induction with reduction of the leaf number to 4 or 5. Further treatment had no additional effect.

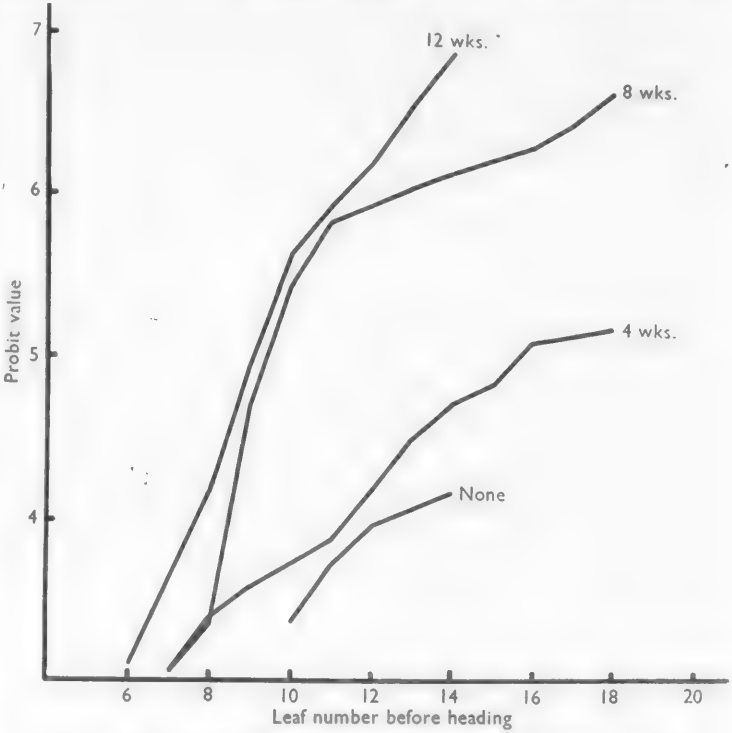


FIG. 4.—Effect of low temperature induction on inflorescence development in Irish perennial ryegrass.

In Irish, a few plants headed without any inductive treatment, but 12 weeks of low temperature alone and 8 weeks of short-day alone were necessary for complete induction in all plants. The combination of short-day and low temperature was slightly more effective than low temperature alone, but not as effective as short-day alone. Unlike Wimmera, even after 12 weeks' short-day plus cold, the leaf numbers in Irish were not uniform, but ranged from 5 to 12, with a mean of 8·5. Following either 8 or 12 weeks' exposure to short-day alone, however, the leaf number after transfer was very uniform, being either 4 or 5.

It seems that even after complete induction with short-day plus cold, there is still genetic variation within the strain for minimum leaf number.

In Kent, only one seedling headed without cold or short-day, and the requirement for complete induction was rather higher than in Irish. The combination of cold and short-day was again more effective than cold alone. As in Irish, even 12 weeks of short-day plus cold gave a range in leaf numbers from 6 to 13 with a mean of 9·3, contrasting with very uniform heading after 12 weeks of short-day alone.

The differences within strains in response to induction are further illustrated in fig. 4 which gives probit diagrams for heading behaviour in Irish ryegrass under cold induction alone. A few plants can initiate heads without any low temperature while others require more than 8 weeks' cold treatment. A similar range occurs in Wimmera and Kent ryegrasses and also for response to short-day induction. Partial induction treatments evidently reveal considerable genetic differences within the populations, in contrast to their more uniform behaviour when fully induced.

While the strains show the same relative behaviour in these two greenhouse tests, the numerical values for the proportion of heading plants and for leaf number before heading are not the same. This applies particularly to the treatments with short-day alone, and differences in greenhouse temperatures during the short-day exposures may be responsible for these differences in response.

These results not only reveal the developmental basis of differences between strains in heading behaviour in the field after spring sowing, but also show that considerable genetic differences in inductive requirement occur within each strain, differences which are not apparent in the usual environment where all material receives enough cold or short-day for complete induction.

## 5. DEVELOPMENTAL RESPONSES : FLORAL INITIATION

In order to assess the requirement for long photoperiods for floral initiation, it is first necessary to ensure that all the material is fully induced. This can be done either by exposing it to winter conditions outdoors, or by giving enough controlled short-day or low temperature for this purpose.

The first of these methods has been used to test the photoperiodic response of Wimmera, Irish and Kent ryegrass. Seeds were sown in autumn 1948, and the seedlings were transferred to natural photoperiods outdoors soon after germination. In January 1949 the following photoperiod treatments were imposed : (*a*) 9 hr. day throughout ; (*b*) 11 hr. day, as soon as the natural day length reached 11 hr. and (*c*) natural day length throughout.

Both leaf number before heading and date of ear emergence were recorded as measures of reproductive development. The results for

all strains are given in table 11, while the differences *within* Irish are further illustrated in fig. 5.

In Wimmera, all plants headed in spring and summer 1949. There was no difference in leaf number, *i.e.* in time of floral *initiation* between natural day length and 11 hr. photoperiod, but the 9 hr. photoperiod resulted in an average delay of just over one leaf stage with a range from 12 to 18 leaves and an increased variance. Ear emergence was delayed

TABLE 11

*Response to photoperiod in strains of* Lolium. *All sown 15th October 1948, overwintered outdoors*

| | | Natural day length | 11 hr. day | 9 hr. day |
|---|---|---|---|---|
| Wimmera . | Percentage heading | 100·0 | 100·0 | 100·0 |
| | Date of heading { Mean | 3·9 May | 4·4 June | 19·4 June |
| | Date of heading { Var. | 16·7 | 45·2 | 177·6 |
| | Leaf no. { Mean | 12·9 | 12·8 | 14·1 |
| | Leaf no. { Var. | 0·81 | 1·17 | 2·94 |
| Irish . . | Percentage heading | 100·0 | 95·0 | 35·0 |
| | Date of heading { Mean | 12·3 May | (11 June) | ... |
| | Date of heading { Var. | 13·0 | ... | ... |
| | Leaf no. { Mean | 12·1 | (11.9) | ... |
| | Leaf no. { Var. | 0·41 | ... | ... |
| Kent . . | Percentage heading | 100·0 | 5·0 | None |
| | Date of heading { Mean | 11·0 June | ... | ... |
| | Date of heading { Var. | 18·2 | ... | ... |
| | Leaf no. { Mean | 14·5 | ... | ... |
| | Leaf no. { Var. | 0·67 | ... | ... |

All figures based on 20 seedlings.

by a month in the 11 hr. photoperiod compared to natural day, an effect presumably on the rate of inflorescence *elongation*. In the 9 hr. photoperiod ear emergence was still further delayed, with a greatly increased variance ; some plants produced heads as rapidly in 9 hr. day as in natural day lengths, while others were delayed for 8-9 weeks. There is evidently considerable genetic range in response to photoperiod within the population, both for floral initiation and for subsequent elongation.

In Irish, all plants produced heads uniformly in natural photoperiods but only 95 per cent. of the seedlings headed when the photoperiod was reduced to 11 hr. although the median leaf number remained unchanged ; the median date of ear emergence was, however, almost a month later than in natural day. Most plants evidently *initiated*

heads as rapidly in 11 hr. day as in natural day lengths, but elongation of the inflorescence was delayed. In the 9 hr. photoperiod, only 35 per cent. of the seedlings headed and even the earliest of these was considerably delayed in both leaf number and ear emergence. In Irish ryegrass also genetic variation in response to photoperiod is carried between plants in the population, but is only revealed at the threshold photoperiods.

In the late-flowering Kent, all seedlings produced heads uniformly in natural photoperiods, but only one plant headed in the 11 hr. photoperiod and none in the 9 hr. The critical day length for nearly

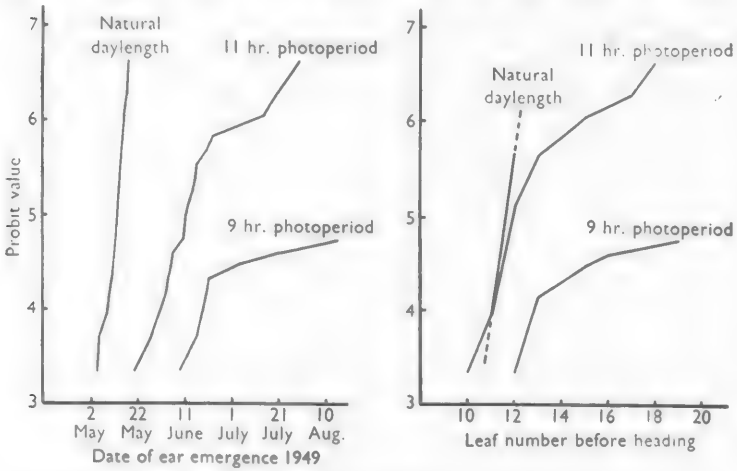


FIG. 5.—Effect of photoperiod on inflorescence development in Irish perennial ryegrass.

all plants is evidently above 11 hr., and the present treatments cannot therefore detect any genetic variation.

These results, while indicating the differences in photoperiodic requirements *between* the three strains, Wimmera, Irish and Kent, also emphasise the genetic variation within the strains, variation which is not apparent in the usual environment but which is revealed at threshold exposures.

## 6. DISCUSSION

The three strains Wimmera, Irish and Kent differ in their heading behaviour in the field, both when autumn and spring sown. These variations in field performance can be traced to genetic differences, firstly in the " winter requirement " for short-day or low temperature before floral induction can occur and, secondly, in the photoperiodic requirements for floral initiation and elongation. These results pose two problems for discussion ; firstly, the relation between the heading

responses of each strain and the climatic and agronomic selection under which it has evolved and, secondly, the maintenance of genetic variation *between* plants in a phenotypically uniform population. These topics will be discussed for each strain separately.

### (i) *Wimmera ryegrass*

Wimmera ryegrass, a winter-annual strain, was first recorded from the Wimmera wheat-growing region of Victoria, Australia (34-36° S.) but it is now grown in West and South Australia and in New South Wales also. It was reputedly introduced from Europe in 1887 (Drake, 1931) and has thus undergone more than 60 generations of selection under Australian conditions. It is similar in morphology and life-cycle to the annual *L. rigidum* from the Mediterranean region, and may have been derived from this source.

The Wimmera region has a semi-arid Mediterranean climate with a January temperature of 60° to 70° F., a July temperature of around 50° F., and a mean annual rainfall of 10-15 in. mainly in the winter. The photoperiod (civil twilight) varies regularly from a minimum of 10¾ hr. in June to a maximum of 15½ hr. in December. Fig. 6 shows the seasonal distribution of photoperiod and the water-balance (according to Thornthwaite's formula) for a similar climate in Adelaide, Victoria. I am indebted to Dr Melvyn Howe, of the Department of Geography, U.C.W., Aberystwyth, for the water-balance diagram (Howe, 1958).

It is evident that the region has a severe water deficit, lasting from about the end of November to the beginning of May when the autumn rains begin. The growing season, as limited by water supply, thus extends from early May to the end of November. In practice (Donald, 1954) Wimmera ryegrass germinates with the autumn rains, grows vegetatively during the winter and initiates heads before the end of September, *i.e.* in photoperiods of less than 13 hr. Ear emergence follows at the end of October and seed must be formed before the water supply is exhausted at the end of November. The plant then dies, the seed is shed and germinates with the autumn rains in the following May. In this way the strain, although annual, persists regularly from year to year.

This limited growing season selects genotypes in which all tillers become fully induced before the end of September, *i.e.* with mean temperatures rarely less than 50° F. and with photoperiods not much below 11 hr., and which can also initiate heads by this date, *i.e.* in photoperiods less than 13 hr. However, the longer the period of vegetative growth, the more tillers and the more seed will be produced per plant, so one would expect quantitative selection for the later end of this range. This, in fact, corresponds to the range in response to winter induction and to photoperiod which is revealed in the present tests. Selection is evidently not for one sharply defined response, but

for any of a wide combination of flowering responses which allow of survival and perpetuation of the plant.

Similar life-cycles and developmental responses have been recorded by Aitken (1955*a*, *b*) in subterranean clover (*Trifolium subterraneum*)

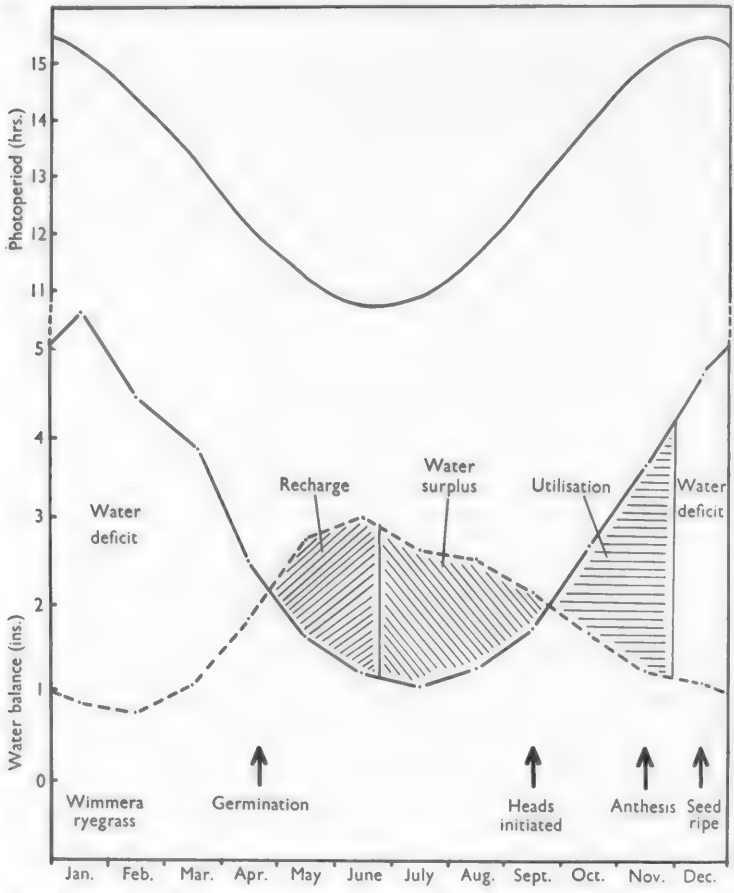


FIG. 6.—Life-cycle of Wimmera ryegrass in relation to water-balance and photoperiod at Adelaide, S. Australia.

—. —. —. —. Potential Evapo-transpiration.
- - - - - - - - - - Precipitation.

which is grown in association with Wimmera ryegrass in the same climatic regions. She has also been able to show that varietal differences in adaptation to the length of the growing season can be related to genetic differences in winter requirement for floral induction and in response to long photoperiod for floral initiation.

### (ii) *Irish perennial ryegrass*

Irish ryegrass is grown for seed in Northern Ireland (55° N.) where more than 100,000 acres are harvested annually. According to Mercer (1929) the growing of ryegrass for seed was introduced about 1830-40 from Ayrshire to Banbridge, County Down. A similar system of management has been maintained throughout; a seeds mixture including 1½ bushels of ryegrass, 10 lb. of cocksfoot and 1 lb. of wild white clover is sown at the end of April or early in May under a cover crop of oats. After the oats are harvested, the sward may be grazed in the autumn, and a seed crop is taken the following summer. The seed harvest nominally begins in the second week in July, but a week to ten days later is more usual. After harvest, the sward is used for grazing or hay, another seed crop being rarely taken. The harvested seed is sold to the wholesale merchant (or since 1946 to the Ministry of Agriculture) who bulks the seed. For the next cycle of seed production the farmer usually obtains seed from the bulked stocks of the seeds merchant. The usual generation time is thus two years, implying about 75 generations of multiplication under the same system of management between 1800 (when seed growing was already well established in Ayrshire), and the present day. Furthermore, the bulking of seed by the merchant means that there has been little opportunity for the development of local populations within the strain.

In Northern Ireland there are no absolute climatic limits to the growing season, and the present heading behaviour has been selected largely by the system of management of the seed crop. In the first place, any plant which produces heads during the summer of the seeding year will shed its seeds and die, or become greatly weakened and not be represented in the subsequent seed crop. Selection will thus operate for a sufficiently high winter requirement to prevent heading after an April sowing. However, the greater the number of fertile tillers per plant in the following spring the greater its representation in the seed crop, so an extremely high winter requirement will also be disadvantageous, and most plants will show a moderate requirement.

Secondly, in the first harvest year, selection will have been for types which give ripe seed at the date of cutting, about the middle of July, corresponding to ear emergence early in May. In Northern Ireland, with a lower spring temperature than Aberystwyth, ear emergence is not likely to occur much before the end of April, however low the critical photoperiod of the material. Consequently, while selection will have set an upper limit to the critical photoperiod, *i.e.* that value which will just give floral initiation in time for emergence at the beginning of May, there will be no such lower limit. This is borne out by the present tests for photoperiodic requirement, where nearly all the plants would initiate heads in an 11 hr. photoperiod, and 35 per cent. would do so even in a 9 hr. photoperiod.

As in Wimmera, it is apparent that more than 70 generations of selection have not produced strict uniformity in developmental

responses within the population, but that a wide range of winter requirements and photoperiodic responses are capable of producing a fairly uniform phenotypic result under the conditions for which the strain has been selected.

### (iii) *Kent perennial ryegrass*

This is a late-flowering pasture strain derived from old pastures in the Romney Marsh and Wealden areas of Kent ; these have in many cases been under grass for several decades and are maintained under intensive grazing management, usually with sheep. Commercial seed is rarely produced directly from these old pastures, but the seed from the old pastures is used to sow down leys, which are themselves grazed intensively for five years before being harvested for seed (Hawkins, 1949). The Kent indigenous seed of commerce is thus two or more generations away from the old pasture. The traditional management varies slightly with the district, but in all cases consists of heavy grazing in the summer followed by light grazing in the winter. In all districts the field is grazed until the end of May before taking the seed crop.

As with Irish, there is little climatic limitation to the life-cycle, and the main selective action has been that of many years of intensive grazing. Under these conditions, only those plants will persist which can maintain themselves indefinitely under heavy grazing, and this implies a high proportion of vegetative to flowering shoots. Jenkin (1930) has shown that plants collected from old grazed pastures are prostrate in growth habit, and usually late-flowering, with little heading in the aftermath, and a high proportion of vegetative to fertile tillers. In the pasture strain, S.23, which is based on such material, Cooper (1951, 1957) has found this lateness of heading to be based on a high critical photoperiod ; similarly the low proportion of fertile tillers results from a high winter requirement and most of the tillers produced in mid to late spring fail to form heads in the same year.

Similar developmental responses are found in Kent ryegrass, the winter requirement for short-day or low temperature being greater than in Irish, and the photoperiodic requirement for floral initiation being higher, resulting in later heading. However, under grazing conditions, there has been no direct selection for uniformity of heading, and late or sparse heading has been a by-product of selection for the maximum production of vegetative tillers. One or two years of harvesting for seed would presumably select for a narrower range of heading dates and the variation in photoperiod response would be correspondingly reduced, particularly as in Kent ryegrass spring temperature no longer limits inflorescence development and the date of heading depends primarily on photoperiod. Seed multiplication would also favour a higher proportion of fertile tillers, and therefore a smaller winter requirement than in the original material.

The variation in date of ear emergence between stocks of Kent

ryegrass from different sources and different generations of multiplication has been studied by Hawkins (1950). He compared the original seed from permanent pastures with that from leys sown with this original seed, and also compared Wealden with Romney Marsh stocks and, as an additional check, with tiller plants collected from a Romney Marsh fattening pasture. The main character recorded was date of ear emergence in overwintered spaced plants. In 1946 and 1947 there were no obvious differences between the stocks from permanent pastures and those from once-grown stocks from leys, although the emergence date of the Wealden series was slightly earlier than that of the Romney Marsh series. In 1948, an early year, however, while all the once-grown stocks from leys were uniform and similar to the original Wealden, those harvested direct from Romney Marsh were more than ten days later than the others, and agreed in emergence date with the tiller plants from the permanent pasture. They also showed a much greater variance *within* stocks. Hawkins (1950) concludes that the grazing management of the leys, particularly grazing up to the end of May before taking a seed crop, is sufficient to maintain the high proportion of vegetative tillers and the lateness of heading of the original permanent pasture stocks.

Again, in Kent ryegrass as in Irish and Wimmera, although the strain is fairly uniform in heading behaviour in the field after overwintering, it is built up of a range of genotypes differing in exact winter requirement for short-day and/or low temperature, and also in the critical long photoperiod for floral initiation. Since heading is late, however, inflorescence development is not limited to any great extent by spring temperature and is under closer photoperiodic control than in Irish; the variation within the strain in response to photoperiod is thus less than in the early strains.

## 7. CONCLUSIONS

The present paper indicates how the range of heading responses found in each strain may have been selected by the combination of climate and management operating in each case. The three strains have evolved in rather different selective backgrounds. In Wimmera, the overriding factor is climatic, the limitation of the growing season by summer drought. This automatically selects for the annual habit, while self-regeneration from seed each year determines the optimum time of ear initiation and seed production in relation to the local distribution of temperature and photoperiod.

In Irish, there are no absolute climatic limitations to growth and that range of flowering responses has been selected which gives the maximum seed yield in the first harvest year under the usual system of management. Such selection largely precludes heading in the seed year, and therefore entails some degree of winter requirement. Since harvesting is comparatively early, the optimum time of heading is

limited by spring temperature, and a wide range of critical photoperiods is allowed.

In Kent, the primary selective effect is that of close and intensive grazing, both in the original permanent pasture and in the leys derived from it. Even when an infrequent seed crop is taken, the field is grazed till the end of May. This intensive grazing management selects for comparatively late heading, and also for a high proportion of vegetative tillers, *i.e.* a high winter requirement. It is not greatly altered by two years' harvest for seed.

In all strains, selection has operated to produce fairly uniform heading behaviour under the conditions of selection. This does not necessarily mean uniformity in developmental responses; in each strain a considerable range of genotypes differing in winter requirement for floral induction, and in critical photoperiod for floral initiation is allowed, provided these give a uniform end-product, *i.e.* date of ear emergence. This genetic variation between plants in developmental responses can often be revealed by altering the exposures to photoperiod and low temperature. A change in the environment may thus reveal genetic variation for selection to work on and produce a new population adapted to the new environment.

A similar range of genetic variation within phenotypically uniform local populations has been reported by Olmsted (1945) in *Bouteloua gracilis*, and by McMillan (1956*a*, *b*) in several other range grasses. While the flowering behaviour of each local population is closely adapted to local climatic conditions, considerable genetic variation is revealed when the population is grown at other climatic locations, or under artificially controlled photoperiods. Similarly, in the self-fertilising subterranean clover, Morley and Davern (1956) find that genotypes which flower at the same date at one station, need not necessarily do so in another environment.

In the present outbreeding strains of *Lolium*, genetic variation is also carried *within* plants in the heterozygous condition, and this serves to replenish the free variation between individuals in each generation. The extent of this potential genetic variation and its rate of release under selection will be discussed in a later paper.

## 8. SUMMARY

1. The aim of this work is a study of the genetic structure of three ryegrass populations, Wimmera, Irish and Kent, paying special attention to the amount of genetic variation carried within small groups of plants and its rate of release under selection. The main character studied is the time and extent of inflorescence development.

2. The present paper describes the genetic variation in heading behaviour between and within these populations after autumn and spring sowing in the field and examines the developmental responses to temperature and photoperiod responsible for these differences in field behaviour.

3. In overwintered material, the date of ear emergence is fairly uniform within each strain, Wimmera and Irish heading in early May, and Kent at the end of May. Year-to-year variation is greatest in the early strains, and can be attributed largely to spring temperature once the critical photoperiod has been reached. Most of the variation in heading date within each strain is genetic ; small differences may occur between stocks in both Irish and Kent ryegrass.

4. Spring sowing delays heading in all strains. In Wimmera, all seedlings produce heads even when sown in April, but with a greatly increased variance. In Irish and Kent, a progressive delay in sowing after January increases the proportion of non-heading plants, and after a critical sowing date (late March for Kent and mid-April for Irish) hardly any plants produce heads. There is a considerable range within each strain in the rapidity with which floral induction occurs after a spring sowing.

5. Greenhouse tests show that the annual Wimmera ryegrass has no obligatory " winter requirement " for short-day or low temperature before floral induction, but that heading is accelerated in many plants by either short-day or cold treatment. In Irish and Kent ryegrasses, few plants can produce heads without short-day or low temperature, the requirement for Kent being greater than for Irish. Considerable variation occurs within each strain in the response to short-day or low temperature.

6. The photoperiodic requirement for floral initiation also varies between and within strains. In Wimmera, all plants will initiate heads in a 9 hr. photoperiod, although ear emergence is delayed at this level ; in Irish, some plants will form heads in a 9 hr. photoperiod, but most require 11 hr. or more ; and in Kent, nearly all plants require more than 11 hr.

7. The range of developmental responses within each strain has been limited by the selective action of the environment. In Wimmera, the main factor is climatic, the restriction of the growing season by summer drought. In the perennial strains, management is more important, the limiting factors being many generations of seed growing in Irish ryegrass, and the effects of intensive grazing in Kent.

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# SOME EVIDENCE CONCERNING THE "MUTATIONAL LOAD" IN INBRED STRAINS OF MICE

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## 1. INTRODUCTION

RADIATION genetics theory postulates that the reproduction of any species of animal is adjusted to the spontaneous mutation rate in that species, and consequently that increases in the mutation rate may, by increasing the "mutational load", jeopardise the survival of the species (Muller, 1954). Hence in studying the radiation genetics of any animal it is essential to know something of the spontaneous mutation rate. The present paper describes an attempt to measure the spontaneous occurrence of recessive lethals in inbred strains of mice.

It is well known that as a result of death of embryos not all the ova shed by a female mouse are represented by living young at birth. McDowell (1924) counted the number of corpora lutea in pregnant mice and the number of young which came to term. He found that the number of living young at birth was only 66 per cent. of the number of corpora lutea. Similarly, Fekete (1947) showed that in the DBA and C57Bl strains the mean litter-sizes at birth were only 58 per cent. and 84 per cent. respectively of the mean number of fertilised ova.

The causes of this death must include :

(i) dominant lethals arising in female gametogenesis ;
(ii) dominant lethals arising in male gametogenesis ;
(iii) recessive lethals ;
(iv) non-genetic agencies.

Of these, deaths due to recessive lethals are only likely to occur with an appreciable frequency when the parents are related, as for instance in an inbred strain. However, it has usually been supposed that the greater part of embryonic death, even in an inbred strain, is due to the fourth cause, *i.e.* to some unfavourable factor of the uterine environment. On the other hand, Hartman (1953), showed that in the opossum defective embryos could be found at a stage so early that their defects were most unlikely to be due to an unfavourable uterine environment.

In the present work crosses were made within and between three inbred strains of mice in all reciprocal combinations, and embryonic death between implantation and 13½-15½ days' gestation was measured by opening pregnant females. The results show that, as might be expected, a proportion of the embryonic death in inbred strains is

traceable to the action of recessive lethal genes, and from the observations an estimate has been made of the mutation rate to such recessive lethals in the strains used. Negative observations of visible mutation in the CBA/H strain are also reported.

## 2. STOCKS AND METHODS

The three inbred strains used were CBA/H, C3H/HeH, and 101/H. Females were used at the age of six weeks to three months, and males at ages of six weeks to four months. The strains were crossed in the nine possible reciprocal combinations, choosing for the within-strain crosses males and females which were sibs. In crosses involving CBA or C3H males, boxes of five females were made up and one male was placed in each box on a Monday morning and left there until Friday morning. In crosses using 101 males, which mate less readily, a male was placed with only one female at a time, the female being replaced as soon as she had mated. Mating was detected by examining females for copulation plugs, on the evening of the first day and the morning of subsequent days. All females which were found to have plugs, and any others which were visibly pregnant, were dissected 13-15 days after mating; the numbers of corpora lutea, of uterine implantations with a live embryo, and of implantations with no live embryo were counted. The crosses were made between September 1957 and May 1958.

## 3. RESULTS

Embryonic loss can be detected in two main ways :

(i) by the difference between the number of corpora lutea, representing the number of ova shed, and the number of implantations in the uterus ; and

(ii) by the number of implantations at which there is no living embryo.

Loss between the shedding of the ovum and implantation could be due either to death of the embryo after fertilisation or to failure of fertilisation. Loss after implantation, however, must involve embryonic death. Hence, in considering the results, pre-implantation and post-implantation loss will be kept separate, and more attention will be paid to post-implantation loss.

Tables 1 and 2 show the ratio of live implants to the total number of implants in each cross. The three lowest ratios are those for the three within-strain crosses, in agreement with the hypothesis that death due to recessive lethals is occurring in these crosses but not, to any appreciable extent, in crosses between strains. When the within-strain and between-strain crosses are summed there is a difference of 9·6 per cent. between the proportions of live implants in the two types, and $\chi^2$ for heterogeneity is 57·5 for 1 degree of freedom. The marginal totals for each type of female and male are given to show that there is no indication of any strain tending to yield a different proportion of dead implants. Moreover, a $\chi^2$ test shows that there is no heterogeneity among the between-strain crosses. Thus there is no indication of any factor other than that being investigated having disturbed the ratios. $\chi^2$ for heterogeneity among the within-strain

crosses is significant at the 1 per cent. level, suggesting that the frequencies of deaths due to recessive effects in the three strains differ. Table 3 shows the values for this excess death in the three strains.

TABLE 1

*Ratio of implantations at which a live embryo was present to the total number of implantations*

| Male parent | Female parent | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|
| | CBA | | C3H | | 101 | | | |
| | Nos. | Per cent. | Nos. | Per cent. | Nos. | Per cent. | Nos. | Per cent. |
| CBA . | 292/336 | 86·9 | 312/341 | 91·5 | 232/254 | 91·3 | 836/933 | 89·6 |
| C3H . | 270/306 | 88·2 | 306/364 | 84·1 | 256/276 | 92·8 | 832/946 | 87·9 |
| 101 . . | 263/285 | 92·3 | 329/350 | 94·0 | 231/309 | 74·8 | 823/944 | 87·2 |
| Total . | 825/927 | 89·0 | 947/1055 | 89·8 | 719/841 | 85·5 | 2491/2823 | ... |

Total within-strain 829/1009, 82·16 per cent.
Total between-strain 1662/1812, 91·72 per cent.

TABLE 2

*$\chi^2$ for heterogeneity*

| | $\chi^2$ | Degrees of freedom | |
|---|---|---|---|
| Between within-strain and between-strain crosses | 57·5 | 1 | $P < 0.001$ |
| Among within-strain crosses . . | 17·7 | 2 | $0.001 < P < 0.01$ |
| Among between-strain crosses . . | 8·5 | 5 | $0.1 < P < 0.2$ |

TABLE 3

*Excess embryonic death in the within-strain crosses for the three strains used*

| Cross | Per cent. survival | Per cent. excess death |
|---|---|---|
| Between-strain . . | 91·7 | ... |
| CBA . . . . | 86·9 | 4·8 |
| C3H . . . . | 84·1 | 7·6 |
| 101 . . . . | 74·8 | 16·9 |

Of all the implants examined 332 had no live embryo. Of these 310 were of the type known as "small moles", which remain when embryonic death has occurred before the formation of the placenta, 8 were "large moles" in which death occurred after the formation of the placenta, but left no recognisable embryo, and at 14 there were

recognisable dead embryos. Four of these 14 occurred in a CBA × CBA cross of one male with his two sisters ; there were 17 implants, 12 with live embryos, 4 with embryos dead at 12-13 days and one small mole. This suggests that in this particular cross a recessive lethal gene causing embryonic death at 12-13 days was segregating. The remaining 18 of the 22 late deaths were distributed more or less randomly.

If the excess deaths were in fact due to recessive lethals, as suggested, rather than to some non-specific effect of inbreeding one would expect variation between females in their numbers of dead embryos according to whether or not a lethal was segregating in that particular mating. Qualitatively, such variation was found, being particularly noticeable

TABLE 4

*Ratios of numbers of implantations to numbers of corpora lutea*

| Male parent | Female parent | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|
| | CBA | | C3H | | 101 | | | |
| | Nos. | Per cent. | Nos. | Per cent. | Nos. | Per cent. | Nos. | Per cent. |
| CBA . | 336/454 | 74·0 | 341/444 | 76·8 | 254/444 | 57·2 | 931/1342 | 69·4 |
| C3H . | 306/377 | 81·2 | 364/445 | 81·8 | 276/384 | 71·9 | 946/1206 | 78·4 |
| 101 . | 285/338 | 84·3 | 350/408 | 85·8 | 303/427 | 71·0 | 938/1173 | 80·0 |
| | 927/1169 | 79·3 | 1055/1297 | 81·3 | 833/1255 | 66·4 | | |

Total within-strain 1003/1326, 75·64 per cent.
Total between-strain 1812/2395, 75·66 per cent.
$\chi^2$ for heterogeneity of within-strain and between-strain crosses is very small.

in the 101 strain, where the amount of death in a few uteri was consistent with the simultaneous segregation of two lethals, whereas in other uteri there were no dead embryos. Statistically, however, the data are not sufficiently extensive for a comparison of the observed and expected distributions of deaths.

Table 4 shows the ratio of implantations to corpora lutea, giving a measure of the pre-implantation loss. In contrast to tables 1 and 2 there is no difference between the two types of cross, within-strain and between-strain. On the other hand the marginal totals for the types of females and males used show large differences. Crosses involving CBA males show more pre-implantation loss than those with C3H or 101 males, and those involving 101 females show more such loss than those involving CBA or C3H females. The cross of CBA male with 101 female has the highest pre-implantation loss of all. Thus there is no evidence in these crosses of recessive lethals tending to cause death of embryos before implantation.

Tables 5 and 6 show the mean numbers of corpora lutea and

uterine implants per female in the various crosses. There are considerable differences among the three strains in the numbers of corpora lutea. Females of strain 101 had a high mean number of corpora lutea and also a high variance of corpus luteum count. Their number

TABLE 5

*Means and standard errors of numbers of corpora lutea per female*

| Male parent | Female parent | | |
|---|---|---|---|
| | CBA | C3H | 101 |
| CBA . . | 9·3 ±0·25 | 10·1 ±0·23 | 13·9 ±0·67 |
| C3H . . | 9·7 ±0·26 | 10·1 ±0·25 | 11·0 ±0·48 |
| 101 . . | 8·6 ±0·31 | 10·5 ±0·28 | 10·4 ±0·52 |
| | 9·20±0·16 | 10·21±0·15 | 11·62±0·35 |

of implants, on the other hand, was average and it appeared that the high corpus luteum count was the result of some females giving a count roughly twice the number of implants. It is suggested that in 101 females developing follicles may be confused with corpora lutea

TABLE 6

*Means and standard errors of number of implants per female*

| Male parent | Female parent | | |
|---|---|---|---|
| | CBA | C3H | 101 |
| CBA . . | 6·9 ±0·31 | 7·8 ±0·38 | 7·9 ±0·32 |
| C3H . . | 7·8 ±0·22 | 8·3 ±0·30 | 7·9 ±0·35 |
| 101 . . | 7·3 ±0·29 | 9·0 ±0·32 | 7·4 ±0·30 |
| Total . | 7·30±0·17 | 8·31±0·20 | 7·71±0·19 |

and that this accounts for their high corpus luteum count and consequent apparent high rate of pre-implantation loss. Among the other types of females there is no reason to suppose that variations in the number of corpora lutea have disturbed the results. In 101 females the count appears to vary with the male used, but since the high numbers of corpora lutea in 101 ovaries were very difficult to count the apparent differences may be due to inaccuracy.

## 4. ESTIMATION OF THE FREQUENCY OF RECESSIVE LETHALS

The increased proportion of dead implants in within-strain crosses could be due to (i) the segregation of clear-cut recessive lethals arising by mutation ; (ii) the segregation of genes with a recessive deleterious

effect arising by mutation and interacting with each other to cause death ; (iii) all embryos of the strain being homozygous for recessive genes which tend to depress their ability to survive in slightly abnormal uterine environments.

If one supposes it to be due to recessive lethals, one can estimate their frequency. If part of it is due to any other cause, the estimate sets an upper limit to the frequency of recessive lethals.

Suppose there are many loci which can mutate to a lethal allele. In some pairs of mice both parents will be heterozygous for one lethal, resulting in the death of one-quarter of their young ; other pairs will be intercross matings for two or more lethals, and others again will not be an intercross for any lethal. Let the sum of the frequencies of intercross matings $(+l \times +l)$ for all these lethals be $m$. Then, if we assume a Poisson distribution, matings which are intercrosses for 0, 1, 2, etc., lethals will occur with frequencies

$$e^{-m}(1, m, \frac{m^2}{2!}, \frac{m^3}{3!}, \ldots)$$

Embryos homozygous for any lethal will die. Therefore the frequencies of surviving embryos will be

$$e^{-m}(1, \tfrac{3}{4}m, \tfrac{9}{16}\frac{m^2}{2!}, \tfrac{27}{64}\frac{m^3}{3!}, \ldots).$$

In the data of table 1 the excess death attributed to recessive lethals was 9·6 per cent. Therefore the survivors were 90·4 per cent.

Hence to find the frequency of intercrosses for a lethal it is necessary to find a value of $m$ for which

$$e^{-m}(1+\tfrac{3}{4}m+\tfrac{9}{16}\frac{m^2}{2!}+\ldots) = 0{\cdot}904.$$

Iteration shows this value to be $m = 0{\cdot}4$.

Haldane (1936) has calculated in terms of the mutation rate the frequency of heterozygotes for a lethal which are likely to be present in an inbred strain as a result of spontaneous mutation. By similar reasoning the frequency of matings which are intercrosses for a lethal can be found. The calculations are shown in the appendix. The result obtained was that if matings which are intercrosses for a lethal leave three-quarters as many young in the succeeding generation as matings not segregating for a lethal, then the frequency of intercross matings is $4n\mu$, where $\mu$ is the gametic mutation rate and $n$ is the number of loci mutating to a lethal.

From the results it has been calculated that the frequency of intercross matings, $m$, is 0·4.

$$\text{Therefore } 4n\mu = 0{\cdot}4$$
$$n\mu = 0{\cdot}1$$

Thus if the excess death in crosses within inbred strains over that in crosses between them is due to recessive lethal genes then any gamete has a chance of 0·1 of carrying a newly mutated recessive lethal. Haldane showed that the frequency of heterozygotes for a lethal in such a strain is $9{\cdot}\dot{3}n\mu$, or in this case $0{\cdot}9\dot{3}$. Hence the results suggest that the frequency of heterozygotes for a lethal in an inbred strain is near unity.

However, this figure is based on the sum of the numbers of live and dead implants over the three strains used, and it has been shown that there is significant heterogeneity among the strains, the CBA strain showing only 4·8 per cent. more dead than in the between-strain crosses and the 101 strain 16·9 per cent. At first sight this seems to imply that in order to continue to accept the hypothesis of recessive lethals one must postulate different mutation rates in the three strains. However, Haldane showed that differences in the method of maintaining the strains could alter the frequency of heterozygotes for a lethal. He considered two possibilities, either that intercross-matings should leave three-quarters as many offspring in the next generation as the other types, or that they should leave an equal number. Another method of stock maintenance, practised in the CBA strain used in this experiment, is to select for production when choosing parents for the next generation. In this strain animals for breeding were chosen from among those whose parents' first two litters were above average in size. The offspring of lethal-intercrosses, which have a reduced litter-size due to death of homozygotes, would thus have a poor chance of being chosen as parents. In the 101 strain, on the other hand, little selection was practised and only a small stock was kept. Since only a few young were kept for breeding from each pair of parents, lethal-intercrosses may have stood an equal chance with the other types of mating of leaving offspring.

The exact chance of a lethal-intercross leaving young in the next generation cannot be decided for either strain but by taking the extreme cases one may find within what limits the observed proportion of dead embryos may vary for a given mutation rate. These extremes are (*a*) that an intercross leaves no offspring and (*b*) that an intercross leaves as many offspring as the other two types in the next generation. It can be shown (see appendix) that the frequencies of intercross matings will be in case (*a*) $2n\mu$ and in case (*b*) $6n\mu$.

Thus if $n\mu = 0{\cdot}1$, in the first case $m$, the frequency of intercross matings, is $2n\mu$

$$m = 2n\mu$$
$$= 0{\cdot}2$$

And in the second case

$$m = 6n\mu$$
$$= 0{\cdot}6$$

The proportion of surviving animals in the strain is given by

$$S = e^{-m}(1+\tfrac{3}{4}m+\tfrac{9}{16}\frac{m^2}{2!}+\tfrac{27}{64}\frac{m^3}{3!}+\ldots).$$

If $m = 0{\cdot}2$ $\quad S = 0{\cdot}951$
If $m = 0{\cdot}6$ $\quad S = 0{\cdot}861$

Thus, with $n\mu = 0{\cdot}1$ the proportion of recessive lethal death could range from 4·9 per cent., a value close to that in the CBA stock, to 13·9 per cent., rather less than that in the 101 stock. Therefore, allowing for sampling error, the results are not incompatible with the hypothesis that in all three strains recessive lethal genes arise with a frequency of 0·1 per gamete.

The spontaneous mutation rate at 7 loci mutating to recessive visible mutations in the mouse is of the order of $1\times10^{-5}$ per locus in male gametogenesis (Russell, 1951; Carter, Lyon and Phillips, 1958). If the average mutation rate to lethal alleles were of the same order

$$n\mu = 0{\cdot}1$$
$$n = 0{\cdot}1\times10^{5}$$
$$= 10\times10^{3}$$

*i.e.* the number of loci which mutate to a lethal in the mouse would have an upper limit of the order of ten thousand.

## 5. VISIBLE MUTATIONS

In the maintenance of the CBA stock in this laboratory during the three years from April 1955 to April 1958 no dominant or recessive visible mutation has been observed. From this negative observation it is possible to find an upper limit to the mutation rate to these classes of genes.

### (i) *Dominant autosomal visibles*

During the three years 25,000 young CBA mice were reared to weaning age and dominant visible mutations producing a clear effect could have been observed in them. In fact none was found. By scanning the Mouse Mutant Gene Symbols list in *Mouse News Letter, 19*, it was found that dominant visible mutations have so far been observed at 32 loci. If the average mutation rate to dominant visibles is $1\times10^{-5}$ and these 32 known loci are potentially mutating, then one would expect to find 32 mutant young per 100,000 tested gametes. Observing 25,000 young is equivalent to observing 50,000 gametes and one would therefore expect 16 mutant young. Clearly the average mutation rate to dominants must be less than $1\times10^{-5}$. The observed mutation for the 32 loci is 0/50,000, which has an upper fiducial limit at the 5 per cent. level of 3·69/50,000. (Fisher and Yates, 1953, table 8 : 1). This is $7{\cdot}38\times10^{-5}$ for the 32 loci or $2{\cdot}3\times10^{-6}$ per locus. Thus if the 32 loci known so far were the only loci in the mouse at which

dominant visible mutations could occur then the maximum compatible average mutation rate to autosomal dominants would be $2{\cdot}3\times10^{-6}$. In fact it is unlikely that all loci at which dominant mutations could occur have already been discovered, so the average mutation rate is likely to be correspondingly lower.

### (ii) *Recessive autosomal visibles*

Recessive visible mutations will only be detected when both parents are heterozygous. Hence, the calculation of the mutation rate is similar to that for recessive lethals. In this case intercrosses for a visible mutation, if found, would be removed from the stock and not permitted to leave young as parents of the next generation. Hence, the case corresponds to that case in the consideration of recessive lethals in which lethal-intercrosses left no young, *i.e.* the frequency of intercross matings is $2n\mu$.

During the three years under consideration 1500 pairs of CBA mice reared young to weaning age. No pair segregated for a recessive visible. Hence the upper fiducial limit of the frequency of intercrosses is 3·69/1500 (Fisher and Yates, 1953, table 8 : 1). From *Mouse News Letter, 19*, the number of known loci mutating to recessive visibles is 100.

$$\begin{aligned} 2n\mu &= 3{\cdot}69/1500 \\ 200\mu &= {\cdot}00246 \\ \mu &= {\cdot}0000123 \\ &= 1{\cdot}23\times10^{-5} \end{aligned}$$

*i.e.* if there were no more than 100 loci mutating to recessive visibles in the CBA stock the maximum compatible mutation rate per locus would be $1{\cdot}2\times10^{-5}$. In fact, as with the dominant visibles, there are likely to be more than 100 loci with the average mutation rate again correspondingly lower. It thus seems probable that the seven loci studied by Russell (1951) and Carter, Lyon and Phillips (1958) and found to have an average mutation rate of $1\times10^{-5}$ are rather more spontaneously mutable than the majority of loci mutating to recessive visibles.

## 6. DISCUSSION

The results of this investigation into the causes of embryonic death in inbred strains do not show whether the excess death within a strain is due to mutations to recessive lethals or to some other cause covered by the term "inbreeding depression". It is known that tendencies to particular lethal abnormalities can occur in particular strains, *e.g.* the persistent low frequency of harelip and cleft palate in strain A. It is therefore reasonable to suppose that tendencies to early embryonic death may also occur and that not all the excess deaths within strains are due to recessive lethals. On the other hand, as females were opened at $13\frac{1}{2}$-$15\frac{1}{2}$ days' gestation, lethals acting late in embryonic life or after

birth were not detected. But late embryonic death seems relatively uncommon in the mouse and so it is unlikely that this will have affected the results appreciably. Hence the estimate of 0·1 for the frequency of newly arisen lethal mutations in the mouse can be taken as an approximate upper limit. The incidence of newly arisen autosomal recessive and sex-linked lethals in *Drosophila melanogaster* is of the order of 0·01 (Muller, 1954), hence the frequency of spontaneous lethals in the mouse may be up to ten times that in *Drosophila*. The figure obtained for the number of loci mutating, 10,000, when the average mutation rate to lethals is taken as $1 \times 10^{-5}$, is in line with expectation. Carter (1957) suggested, as a result of studying the induction of recessive lethals by $\gamma$-irradiation in the mouse, that the number of mouse loci was likely to be not very different from that in *Drosophila*, for which a figure of 5000-10,000 is usually given.

Comparison with the negative observations on dominant and recessive visible mutation suggests that mutation to lethals is likely to occur more frequently than mutation to visibles in the mouse. The upper limit of the average mutation rate to dominants is less than the average mutation rate observed in other experiments to seven recessive visibles. Mutation to dominants is thus probably less common than mutation to recessives. Both these facts are in accordance with findings in *Drosophila*.

## 7. SUMMARY

Embryonic death has been measured in the nine possible reciprocal crosses within and between three inbred strains of mice, CBA/H, C3H/HeH and 101/H.

Post-implantation death was higher in the within-strain than in the between-strain crosses, the excess death being 4·8 per cent., 7·6 per cent. and 16·9 per cent. in strains CBA, C3H and 101 respectively, and 9·6 per cent. when summed over all crosses. Some, but probably not all, of this death is likely to be due to the segregation of recessive lethals arising by mutation in the strain.

Assuming all excess death to be due to recessive lethals an upper limit to the spontaneous mutation rate to recessive lethals in these strains was calculated. This limit is 0·1 per gamete over all loci, giving an estimate of 10,000 loci mutating to lethals if the average mutation rate per locus is $1 \times 10^{-5}$.

In the routine maintenance of the CBA stock during a period of three years no dominant or recessive visible mutations were found. From this negative observation it is possible to calculate upper limits to the mutation rates per locus to dominant and recessive visibles. These limits are, for recessive visibles, $1 \cdot 2 \times 10^{-5}$ if 100 loci are mutating and, for dominant visibles, $2 \cdot 3 \times 10^{-6}$ if 32 loci are mutating. The numbers of loci given are those which are so far known ; if as is likely the total numbers of loci are greater than these then the upper limits of the mutation rates will be lower.

*Acknowledgment.*—The author is grateful to Miss A. K. Fenn for technical assistance.

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## 9. APPENDIX

### (i) *Frequency of intercrosses for a lethal*

Let $a$ be a recessive lethal gene and let matings of types $AA \times AA$, $AA \times Aa$, and $Aa \times Aa$ occur with frequencies $x_n, y_n$, and $z_n$ in the $n$th generation after the first mating involving a mutant heterozygote. Then, if $Aa \times Aa$ matings leave $\frac{3}{4}$ as many young in the succeeding generation as the other two types, it can be shown that

$$x_{n+1} = x_n + \tfrac{1}{4} y_n + 1\tfrac{1}{2} z_n$$
$$y_{n+1} = \tfrac{1}{2} y_n + \tfrac{1}{3} z_n$$
$$z_{n+1} = \tfrac{1}{4} y_n + \tfrac{1}{3} z_n$$

The first, $x_0$, generation will consist of a $y$-mating with a frequency of 1.

Thus the first few terms of the generation series for each type of mating will be

$$x \quad 0, \tfrac{1}{4}, \tfrac{1}{2}, \tfrac{11}{16}, \ldots$$
$$y \quad 1, \tfrac{1}{2}, \tfrac{1}{3}, \tfrac{17}{72}, \ldots$$
$$z \quad 0, \tfrac{1}{4}, \tfrac{5}{24}, \tfrac{11}{72}, \ldots$$

From these terms the recurrence relation for $z$ in successive generations has been calculated using the equations

$$u_2 = bu_1 + cu_0$$
$$u_3 = bu_2 + cu_1$$

where $u_0 \ldots u_3$ are the first few terms of the series for which the recurrence relation is required, and $b$ and $c$ are the coefficients to be found (Li, 1955, p. 66).

It is shown to be

$$12z_{n+2} - 10z_{n+1} + z_n = 0.$$

From this relation the frequency of $z$-matings over all generations has been summed to infinity, using the formula given by Haldane (1936). In this case the sum to infinity is 1. Since the chance of occurrence of a $y$-mating in the first place is $4\mu$, where $\mu$ is the gametic mutation rate, the frequency of $z$-matings over the whole inbred strain is $1 \times 4\mu$, or if there are $n$ loci concerned, $4n\mu$.

### (ii) *Frequencies of intercrosses under other systems of stock maintenance*

The two cases to be considered are (*a*) that $z$-matings leave no offspring in the succeeding generation, and (*b*) that $z$-matings leave as many offspring as the other two types.

For the first case the frequencies of mating types will be given by

$$x_{n+1} = x_n + \tfrac{1}{4}y_n$$
$$y_{n+1} = \tfrac{1}{2}y_n$$
$$z_{n+1} = \tfrac{1}{4}y_n.$$

The first few terms will be

| | | | | |
|---|---|---|---|---|
| $x$ | 0, | $\frac{1}{4}$, | $\frac{3}{8}$, | $\frac{7}{16}$ |
| $y$ | 1, | $\frac{1}{2}$, | $\frac{1}{4}$, | $\frac{1}{8}$ |
| $z$ | 0, | $\frac{1}{4}$, | $\frac{1}{8}$, | $\frac{1}{16}$. |

The recurrence relation for $z$ is

$$2z_{n+2} - z_{n+1} = 0$$

and the sum to infinity is $\frac{1}{2}$.

Therefore the frequency of $z$-matings in the inbred strain is $\frac{1}{2} \times 4n\mu$ or $2n\mu$. Similarly, the frequency of heterozygotes for a lethal, which is equal to $(\frac{1}{2}y+z)$, is $6n\mu$.

For the second case the frequencies of matings are given by

$$x_{n+1} = x_n + \tfrac{1}{4}y_n + \tfrac{1}{16}z_n$$
$$y_{n+1} = \tfrac{1}{2}y_n + \tfrac{1}{8}z_n$$
$$z_{n+1} = \tfrac{1}{4}y_n + \tfrac{1}{8}z_n.$$

The first few terms are

| | | | | |
|---|---|---|---|---|
| $x$ | 0, | $\frac{1}{4}$, | $\frac{29}{72}$, | $\frac{673}{1296}$ |
| $y$ | 1, | $\frac{1}{2}$, | $\frac{13}{36}$, | $\frac{185}{648}$ |
| $z$ | 0, | $\frac{1}{4}$, | $\frac{17}{72}$, | $\frac{253}{1296}$ |

and the recurrence relation for $z$-matings is

$$18z_{n+2} - 17z_{n+1} + 2z_n = 0.$$

The sum to infinity is $1{\cdot}5$ and hence the frequency of $z$-matings in the strain is $6n\mu$. Haldane has shown that the frequency of heterozygotes in a strain maintained in this way is $12{\cdot}6n\mu$.

# AN EVOLUTIONARY STUDY OF THE BUTTERFLY *MANIOLA JURTINA* IN THE NORTH OF SCOTLAND

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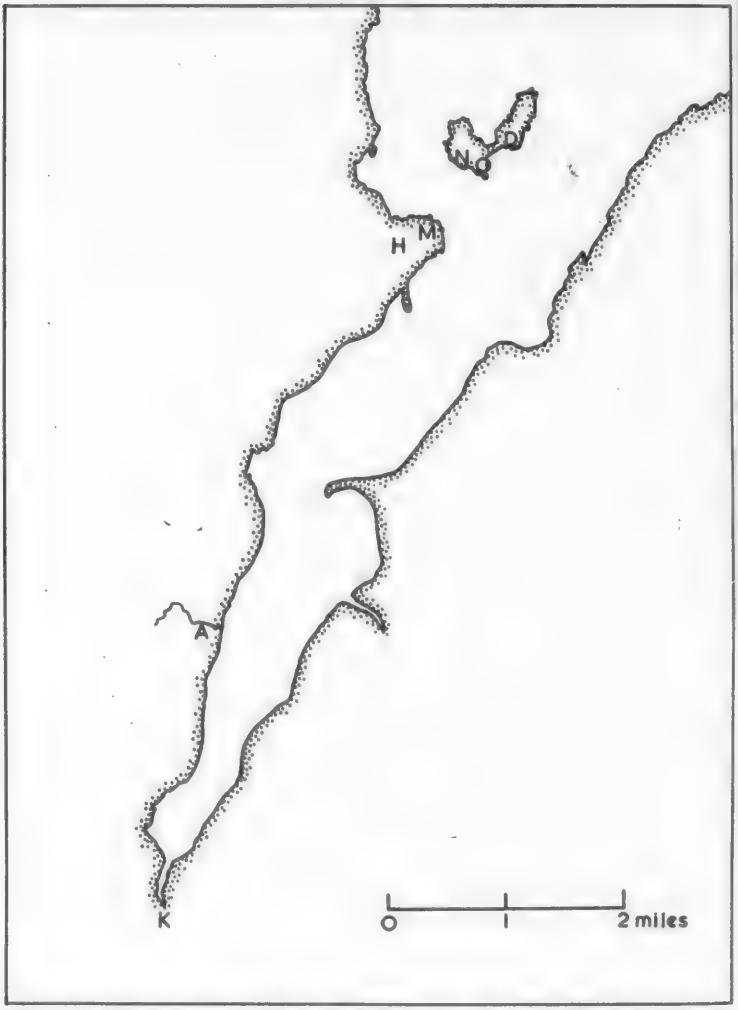
A SERIES of studies on the butterfly *Maniola jurtina* (Satyridæ) has demonstrated that the distribution of spotting on the underside of the hind wings can be used as a measure of evolutionary divergence between colonies of that species (Dowdeswell, Fisher and Ford, 1949 ; Dowdeswell and Ford, 1952, 1953, 1955 ; Dowdeswell, Ford and McWhirter, 1957 ; McWhirter, 1957). This work has been carried out in the Isles of Scilly and the southern half of England, augmented by a few samples from Ireland and the Isle of Man. In July 1956, one of us (Bruce Forman) with a party of students from the University of Aberdeen, visited Sutherland with the object of extending these investigations to a district in the far north. The samples so obtained were scored and analysed by E. B. F. and K. G. McW., so that the results should be strictly comparable with those published previously. The success achieved by the party was largely due to the care which they took to provide full and accurate observations on the habitats of the insect.

## 1. DESCRIPTION OF THE LOCALITIES

The work was carried out on the North Sutherland coast, on Rabbit Island (National Grid NC/602631 *) and on the mainland opposite ; see the map, in which the various localities are indicated by the letters used in this account. Three colonies of sufficient size for sampling were found on the mainland. The most southerly of them (K), near Kinloch Lodge, inhabited open spaces among birch woods. It was rather over an acre in extent and was traversed by the road from Tongue to Durness, which was no barrier to the butterfly. Part of the area had been a hay field and part was somewhat boggy. It was separated by two and a half miles from locality A. This comprised slightly less than half an acre along either bank of the Allt Loch Fhionnaich within a little gorge which is also crossed by the Tongue-Durness road. The sides of this gorge were occupied by trees, with open grassy spaces and some bracken. The whole was surrounded by moorland except at the eastern end where, north of the stream, a patch of bracken extended from the road down to well-cropped grass reaching to the shore.

* 4°, 24′, 3″ west ; 58°, 32′, 4″ north.

The third mainland locality (M) was approximately four miles farther to the north-east, principally over moorland. It consisted of a number of gullies separated by low ridges running down to the shore ;




Map showing Kyle of Tongue, North Sutherland coast, Scotland, with Rabbit Island (National Grid NC/602631). Localities are indicated by the letters used in the text.

in all, about an acre. Here the grass was short, with scattered thistles and interspersed with considerable patches of bracken.

In addition, a few specimens were obtained in a hay field (H) about one-third of a mile west-south-west of M and separated from it

by higher ground with short turf where no butterflies were seen. However, the insects were here so rare that no effective sample could be obtained.

There can be little doubt that the principal mainland localities (and indeed that at H) are isolated from one another ; for the butterfly seems absent from the intervening country, most of which appears to be quite unsuited to it, while in the circumstances the distances involved are far too great for it to traverse. Indeed repeated observations on the Isles of Scilly have shown that 150 yards of unsuitable terrain form an effective barrier to this insect, though it can, and constantly does, travel farther than this in a few minutes within its own localities ; so demonstrating the importance of minor ecological barriers to this, and we believe to many other, species.

Locality M is the nearest point on the mainland to Rabbit Island, from which it is separated by a channel, 1000 yards across, consisting of tidal sand and a narrow strip of permanently open water. This island is in two parts, connected only by a bar of sand with some rocks, approximately 300 yards long, which remains uncovered at high tide. Upon the south-western portion, being that nearer the mainland, are two colonies of *Maniola jurtina* at N and O (see map). That at N faces across the channel to locality M and occupies about an acre. It is in the form of two gullies meeting behind a mound. The vegetation consists of grass, with bracken, nettles, thistles and primroses. To the east it is separated by a sandy valley, 10-20 feet deep and 20 yards wide, from area O. This is an irregular peninsula, of perhaps half an acre, with short turf and patches of bracken and primroses.

Colonies N and O are much too close to be isolated from one another. The rest of this part of the island is higher, relatively flat and exposed and, though not examined in detail, it was clear that the butterfly was not established upon it.

The north-eastern portion of Rabbit Island contained one colony only (D) of *Maniola jurtina*, at the end of the connecting sand bar. At this point two shallow gullies running inland from the shore meet behind a hillock, providing a locality very similar in shape and vegetation to that at N. Only a few scattered specimens were found farther on. This colony is almost certainly isolated from those at N and O, being separated from them by 300 yards of sand, with a further 100 yards of unsuitable terrain (poor grass on sand from which the butterfly is absent) before reaching colony O.

## 2. THE SUTHERLAND DATA AND THEIR ANALYSIS

The results of scoring the spot-numbers of the butterflies collected in each locality are shown separately for the two sexes in tables 1 and 2. A few additional specimens were obtained which were too worn to evaluate.

The five specimens captured in locality H (four males and one female) must be omitted, for it would be prejudicing the situation to combine them with the nearest effective sample (from M) since the

TABLE 1

*Male spot-distributions per hind wing*

| Locality | Spot numbers | | | | | Totals |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | |
| Mainland, K . . . | 1 | 9 | 20 | 3 | 1 | 34 |
| ,, A . . . | ... | 8 | 17 | 1 | 2 | 28 |
| ,, M . . . | 3 | 2 | 17 | 8 | ... | 31 |
| ,, H . . . | ... | 1 | 3 | ... | ... | 4 |
| Rabbit Island, N . . . | 3 | 6 | 38 | 4 | 2 | 53 |
| ,, ,, O . . | 1 | 1 | 9 | 3 | 1 | 15 |
| ,, ,, D . . . | 2 | 18 | 33 | 2 | ... | 55 |
| | 10 | 46 | 137 | 21 | 6 | 220 |

distance between the two places is certainly sufficient to provide isolation. We can therefore consider the three principal mainland samples, those from localities K, A and M. When testing the males for homogeneity the numbers are so small that only two groupings

TABLE 2

*Female spot-distributions per hind wing*

| Locality | Spot numbers | | | | | Totals |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | |
| Mainland, K . . . | 21 | 8 | 3 | ... | ... | 32 |
| ,, A . . . | 15 | 6 | ... | 1 | ... | 22 |
| ,, M . . . | 14 | 7 | 2 | ... | ... | 23 |
| ,, H . . . | ... | ... | 1 | ... | ... | 1 |
| Rabbit Island, N . . . | 17 | 4 | 1 | 1 | ... | 23 |
| ,, ,, O . . | 6 | 1 | ... | ... | ... | 7 |
| ,, ,, D . . . | 10 | 5 | ... | ... | ... | 15 |
| | 83 | 31 | 7 | 2 | ... | 123 |

of spots, $<2$ and $2+>2$ can be used. This gives $\chi^2_{(2)} = 1{\cdot}02$, and $P$ between $0{\cdot}7$ and $0{\cdot}5$. Similarly, the numbers are insufficient for more than two spot-groupings for the females ; 0 and $>0$, for which $\chi^2_{(2)} = 0{\cdot}27$ with $P$ between $0{\cdot}9$ and $0{\cdot}8$. Thus, though the colonies in these localities are isolated from one another, there is no indication in either sex of heterogeneity between them.

One of the two populations on the south-western portion of Rabbit Island, that from locality O, is too small for separate treatment and, as it certainly cannot be isolated from that at N, these are grouped

together. When they are compared with the one at D, in the north-eastern division, a significant difference is found in male spotting, $\chi^2_{(2)} = 9{\cdot}12$ and $P$ is 0·02 to 0·01. The number of females is so small that a considerable difference might exist between their spot-distributions on the two parts of Rabbit Island without being revealed by the present data. As these stand, $\chi^2_{(1)} = 0{\cdot}13$ so that $P$ is approximately 0·7 and they are not heterogeneous. Clearly, however, having regard to the male situation, the reality of the female homogeneity cannot be assumed.

When we compare the total mainland captures, already shown homogeneous, with the total from the south-western part of Rabbit Island, we find substantial homogeneity for the entire assemblage in both sexes. The males provide a sufficient sample for a comparison using $<2$, 2 and $>2$ spots. Here $\chi^2_{(2)} = 2{\cdot}38$, with $P$ lying between 0·5 and 0·3. The females can only be treated as individuals with 0 spots and those with 1 or more. They give $\chi^2_{(1)} = 0{\cdot}88$ * with $P = 0{\cdot}5$ to 0·3.

There is thus evidence that in *Maniola jurtina* the three populations from the Scottish mainland, and that from the combined localities N and O on the nearer (south-western) part of Rabbit Island, are stabilised to a similar spot-distribution respectively in the two sexes, although the four habitats are isolated from one another. On the other hand, the adjustment of the butterfly to the conditions on the farther (north-eastern) section of Rabbit Island is significantly different as judged by male, but not by female, spotting.

## 3. COMPARISON WITH MATERIAL FROM OTHER LOCALITIES

### (i) *The females*

Up to 1952 the female spot-distributions of *Maniola jurtina* were of a constant pattern, with a single large mode at 0 spots, in all the Southern English localities so far studied from west Devon to the east coast. These include the transition from an Atlantic to a semi-Continental climate, alkaline and acid soils, with their widely distinct ecologies, and the vegetation both of poor downland and of rich meadows. It is evident, therefore, that in this region female spotting is stabilised to widely diversified conditions. Yet at the Devon-Cornwall border it changes abruptly to a different form : a bimodal one with a larger mode at 0 spots and a smaller at 2. Farther west in Cornwall, and on the Isles of Scilly, populations with other and highly characteristic spot-distributions are found (Dowdeswell and Ford, 1953).

A more detailed analysis, however, showed that within the unimodal type of female spotting east of the Devon-Cornwall border, two subdivisions could be recognised. A more general one, the " Old English ", with a relatively large mode at 0 spots, comprising 60 per

* Yates' correction is used in this and all other $\chi^2$ values with a single degree of freedom.

cent. of the specimens or more, and another, the "New English", found in a few places only, in which the single mode at 0 is less pronounced, comprising 50 per cent. of the specimens or less.

By 1956 the "New English" type had become more general in the region from Devon to the east coast, though some populations remained "Old English", while in a few instances a new spot-distribution, the "Pseudo-Cornish", had appeared. This has a second small mode at 2 spots in the females, reminiscent of the East Cornish

TABLE 3

*Totals for Sutherland localities, K, A, M, N+O, combined (excluding D)*

| Spots . . | 0 | 1 | 2 | 3 | 4 | Total |
|---|---|---|---|---|---|---|
| Male . . | 8 | 27 | 101 | 19 | 6 | 161 |
| Female . . | 73 | 26 | 6 | 2 | ... | 107 |

type, which had not itself altered so far as our information extends. There is evidence that, in general, *M. jurtina* is now tending to return to the "Old English" state, with a single large mode at 0 in the females.

As already indicated, we have in the Sutherland data now available a stabilisation which transcends the considerable isolation of localities

TABLE 4

*Southern English specimens from three localities (see text), 1956*

| Spots . . | 0 | 1 | 2 | 3 | 4 | Total |
|---|---|---|---|---|---|---|
| Male . . | 6 | 22 | 133 | 44 | 9 | 214 |
| Female . . | 191 | 84 | 35 | 5 | 2 | 317 |

K, A, M and N+O from one another. The combined spot-frequencies (already shown homogeneous within each sex) are given in table 3. It is surprising here to find, five hundred miles or so farther to the north, a situation characteristic of the greater part of Southern England, though that changes suddenly at the Devon-Cornwall border. For it will be seen that, in Sutherland, female spotting is unimodal at 0 and is of the "Old English" form, since specimens with 0 spots comprise 68 per cent. of the total.

This far northern collection may be compared with the south English samples of the "Old English" type captured the same year (1956), see table 4. These are from three localities, Middleton East (Hampshire), Woolbury Ring (Hampshire), Newton Abbot (Devon). Their female spotting is homogeneous ($\chi^2_{(4)} = 6{\cdot}83$, for which $P = 0{\cdot}2$

to 0·1) ; the separate frequencies will be given in full in papers dealing specifically with the present situation in Southern England. These female samples may therefore be combined, and those from Sutherland are consistent with them : $\chi^2_{(2)} = 3{\cdot}23$, with $P$ approximately 0·2.

The female spot-distributions of these Scottish insects are also similar ($\chi^2_{(5)} = 3{\cdot}75$, with $P$ 0·2 to 0·1) to a widespread series of " Old English " samples taken in 1952 at Ipswich, Winchester, Salisbury and Oxford. That group is homogeneous, with $\chi^2_{(6)} = 5{\cdot}90$, giving $P$ from 0·5 to 0·3. (These data are from Dowdeswell and Ford, 1953, table 2, from which Taunton is omitted since it is now known that it was then " New English " and in process of changing to the " Pseudo-Cornish " type.)

### (ii) *The males*

The Southern English males are unimodal at 2 spots. This arrangement is even more constant than is the female one of a single mode at 0, being found so far as we know not only throughout the whole area from the east coast to west Devon, but also in Cornwall and, generally, in the Isles of Scilly. This is the more remarkable since male spotting is nevertheless capable of taking other values : for instance, unimodal at 3 spots in one island in Scilly and bimodal in an Irish locality. However, McWhirter (1957) has shown that significantly distinct male distributions exist within this highly constant general form and that there is a heavily significant correlation between the male and female conditions. Thus as the proportion of spotless females declines from the higher " Old English " value through the " New English " to the " Pseudo-Cornish ", so the proportion of males with less than 2 spots decreases and that with more than 2 increases. Indeed male spotting, though so generally unimodal at 2, is more sensitive to minor evolutionary adjustments than the female, and serves to discriminate populations even within the three main Southern English groupings.

In the " Old English " stabilisation, in which 60 per cent. or more of the females are spotless, the proportion of males with 1 or 0 spots is together greater than, or approximately equal to, that with 3 spots or more. Sometimes, however, the latter group may amount to twice the number of specimens with less than 2 spots. In the " New English ", and still more in the " Pseudo-Cornish " type, this excess at the higher spot values is much accentuated.

It will be seen from table 3 that the Scottish sample is markedly of the " Old English " type in both sexes. The males may be contrasted with those found in the three " Old English " populations captured the same year (1956) in Southern England.* These are homogeneous with one another ($\chi^2_{(2)} = 1{\cdot}97$, for which $P$ is 0·5 to 0·3). A comparison between the Scottish and English populations shows fairly clear male heterogeneity : $\chi^2_{(2)} = 7{\cdot}87$ with $P$ approximately 0·02.

* The individual data are being published separately.

### (iii) *General comparison in both sexes*

Since spotting shows much the greater degree of minor diversity in the male sex, it is not surprising that the spot-frequencies of the Scottish and English populations captured the same year should be significantly different in the males even though they are similar in the females. It will be noticed that the situation in Sutherland represents a slightly more extreme manifestation of the "Old English" type, for the spotless insects comprise 68 per cent. of the females, and males with less than 2 spots are 1·4 times as common as those with more than 2. Indeed in both sexes a progressive gradient towards lower spot-values is detectable from localities arranged in the order: East Cornwall, Southern England (east of Cornwall), Sutherland, the Isle of Man, Galway (Ireland). However, one of the most important discoveries which results from collecting *Maniola jurtina* in Sutherland is the fact that the females there are unimodal at 0 spots. This clearly suggests a similar adjustment over the greater part of Britain, though it is one which can be broken down abruptly when a different type is favoured, as at the Devon-Cornwall border.

## 4. SUMMARY

(1) Samples of the butterfly *Maniola jurtina* have been collected from five localities in North Sutherland. Three are on the mainland and two are respectively on the two parts of Rabbit Island which are connected with one another only by a bar of sand.

(2) The populations in these localities are all isolated from one another. Two habitats (N and O) upon the south-western portion of the island form a non-isolated unit which can be combined. The individuals caught in an additional mainland locality (H) are too few for analysis.

(3) Previous work has proved that spotting in this species, which takes different distributions in the two sexes, provides a sensitive criterion of evolutionary adjustment.

(4) Using that character, the three mainland populations and the one on the nearer (south-western) part of Rabbit Island prove to be homogeneous.

(5) The population on the more remote (north-eastern) part of the island has been independently adjusted to that habitat, being significantly different from the others, judged by the male spot-distribution. Too few females were obtained to provide an effective comparison in that sex.

(6) The males in Sutherland are unimodal for 2 spots like all Southern English communities, including the Cornish localities (which are distinct from the rest of the country in the female sex). Minor but significant differences in the distribution of male spotting occur within this unimodal form, and in this respect the Sutherland males differ significantly also from the Southern English ones.

(7) In certain South English habitats the species has undergone a significant change since 1952 which, indeed, was already apparent in a few places in that year. The Scottish samples represent a somewhat more extreme phase of the former Southern English stabilisation, from which certain of the English populations have not diverged and to which the others appear to be returning.

(8) The Sutherland insects in both sexes tend to conform, though in a less degree, to the type with relatively few spots found in the Isle of Man and in Galway, Ireland.

(9) Female spotting, which takes various distinct forms in Cornwall and the Isles of Scilly, is generally adjusted to a uniform type (unimodal at 0 spots) in Southern England, where it is stabilised throughout a wide range of climatic and ecological differences.

(10) The Scottish samples, though caught five hundred miles farther north, are similarly stabilised, being unimodal at 0 spots in the female. This is the more remarkable since that condition alters abruptly to a bimodal one at the Devon-Cornwall border.

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# EVOLUTIONARY STUDIES ON *MANIOLA JURTINA*: THE ENGLISH MAINLAND, 1956-57

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## 1. DEVELOPMENTS IN THE AREA OF THE SOUTH ENGLISH STABILISATION

In the years 1950-52 Dowdeswell and Ford (1952, 1953) found that throughout a large part of Southern England the females of *Maniola jurtina*, the Meadow Brown Butterfly, were so stabilised that all populations showed a characteristic spot-distribution with a large mode at 0 spots. The area in question stretched from the east coast (Ipswich), westwards to the Devon-Cornwall border, where there occurred a change within some twelve miles to another stabilisation in which the female spot-distribution is bimodal with a large mode at 0 spots and a lesser mode at 2 spots (defined as the East Cornish stabilisation). In their 1953 paper, however, they noted that at Tiverton (1951) in the extreme west of the South English stabilisation area, the female distribution showed a reduction of the steepness of the mode at 0 spots. The sample obtained at Taunton, Somerset, in 1952, had a similar tendency. With the small number of samples then available, however, these slight divergences did not much disturb the extreme and remarkable homogeneity of South English populations up to the region of the Cornish border.

By 1955 two other stabilisation areas, apart from those of South England and East Cornwall had been discovered, one in Penwith (in west Cornwall) and the other in the large islands of the Scilly group.

Sampling in the South English area was resumed in 1956 and it immediately became clear that the old stabilisation had broken up; most populations showed greatly increased spotting in both males and females, and the mode at 0 spots in the female distributions was reduced and even sometimes accompanied by a secondary mode at 2 spots—a condition which up to 1952 had only been observed in east Cornwall and a few adjacent parts of Devon.

### (i) *Definitions of the South English spot-distributions*

For convenience we have named the three types of spot-distribution found in the females in 1956 and 1957 as follows:

(i) *Old English* (O.E.): the females maintain a pattern such that the specimens at 0 spots are 60 per cent. or more of the total

of specimens at 0, 1 and 2 spots, and the specimens at 2 spots do not exceed the number of those at 1 spot.

(ii) *New English* (N.E.) : the females show a pattern in which the specimens at 0 spots are less than 60 per cent. of the total at 0, 1 and 2 spots ; the specimens at 2 spots do not exceed the total of those at 1 spot.

(iii) *Pseudo-Cornish* (P.C.) : the females show a pattern in which there is a major mode at 0 spots and a minor mode at 2 spots.

No attempt will be made here to deal in detail with the male spot-distributions. It may be briefly said that males with spot-numbers in excess of 2 were generally much more common than those with spot-numbers less than 2, and that some correlation with the three grades of female spot-frequency could be seen.

### (ii) *Features of the South English data of* 1956 *and* 1957

All the data for these years are reproduced in the appendices 1 to 4. Three main trends can be observed in them :

(i) 1956 seems to have marked the peak of a wave of high-spotting which occurred simultaneously nearly everywhere in the former South English stabilisation area. The populations in that year were very large. In 1957, after an abnormally mild winter and a prolonged spring drought, the species was much rarer ; the wave of high-spotting receded, except in the south-western counties of Devon, Somerset and Dorset.

(ii) In contrast to the situation in 1950-52, there were marked differences in the spot-distributions from place to place in 1956 and 1957. In both these years, however, a strong stabilisation of " New English " populations was found in the south-western part of the area, in the counties of Devon, Somerset and Dorset. This fortunate event has enabled us to study the transition from unimodal to East Cornish bimodal distributions in great detail (see below, pp. 371-82). Figs. 1 and 2 show by means of triangular graphs the relationships of the South English samples in the two years.

(iii) A new and most remarkable feature of the post-1952 South English collections has been intra-seasonal heterogeneity. We are indebted to J. D. F. Frazer for pointing this out. He collected *M. jurtina* in the 1955, 1956 and 1957 seasons at Burham Down, Kent. (His 1955 collection is the only one known to us to have been taken on the mainland in that year.) These samples show a clear pattern in which both males and females have high spot-averages at the beginning of the emergence, but suddenly change to lower spot-averages towards the middle of the emergence. Frazer kindly sent a copy of his data to us late in the summer of 1956. In 1957 and 1958 we obtained samples

throughout the emergence at a number of localities and certain of these confirmed the Burham Down observations.

During the period of stability in 1950-52 we had obtained samples at various stages of the emergence at different localities and this had not, it seemed, in any way upset the general pattern of marked homogeneity. It occurred to us that the intra-seasonal shift, first observed by Frazer, was associated with changes in stabilisation and might indeed be one of the means by which such changes came about. This was confirmed by two opposing sets of data :

(*a*) Sampling of the Middleton (East) population throughout the 1957 emergence showed us that no intra-seasonal shift occurred there (see appendix 3). This locality is one of the two where no departure from the " Old English " stabilisation has been observed.

(*b*) Data from extensive samples of the Ipswich population, which were kindly sent to us by S. Beaufoy, show that the early emergence in 1956 was of a rather high-spotted N.E. type ; in 1957 the early emergence was similar, but was known to be succeeded by an O.E.-type later emergence ; in 1958 the whole emergence was homogeneous and conformed to the N.E. type.

In selectional terms the intra-seasonal shift suggests that in some populations an ecological factor which normally operates adversely against the high-spotted specimens was lifted simultaneously over large parts of South England during such a time of year as to disturb the balance only of the first half of the emergence ; genes for high spotting might then be perpetuated in larger numbers than usual and would then have the opportunity in some cases to percolate through all or most of the emergence next year. This seems to have happened at Ipswich, eventually producing a population stabilised on the N.E. level instead of the O.E. level observed in 1950-52. In other cases, the move towards high-spotting seems to have been unsuccessful and may have been limited to the early part of the emergence before finally disappearing. (Appendices 2 and 4 contain the data relevant to the intra-seasonal shift.)

All data given in this series of papers are the result of estimates made by at least two of the authors according to the system described in Dowdeswell and Ford (1953) ; it would therefore be improper to make direct comparisons between data resulting from scoring by another worker and our data, since slight differences in convention might occur and upset the accuracy of the estimate. We have for this reason listed Frazer's data separately, which he kindly permits us to reproduce, though it is clear that comparisons made between his estimate of spotting in the early and in the later parts of the emergence are perfectly valid.

### (iii) *Geographical patterns in the former South English stabilisation area, 1956-57*

If the former South English stabilisation area is broken up into three sub-areas, (i) the south-western counties, that is, Devon, Somerset and Dorset, (ii) the south-eastern counties, that is, Hampshire, Sussex and Kent and (iii) counties lying north of the line joining the Severn and Thames estuaries, we find that populations in the south-western sub-area, with very few exceptions, have been stabilised on the New

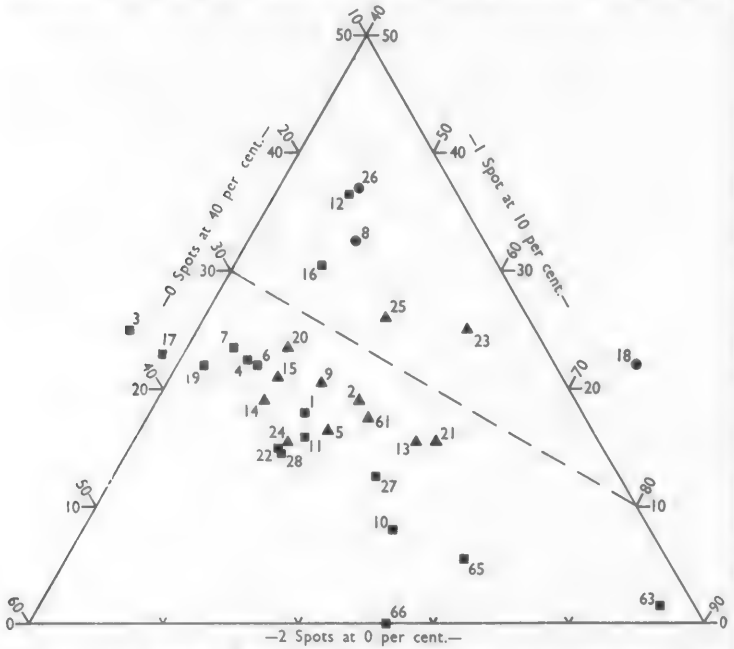


FIG. 1.—*Maniola jurtina*: English mainland and peripheral populations—female spot-distributions 1956. The frequency of 0, 1 and 2 spots are expressed as percentages of the total of these three values, the tail being disregarded. Entries above the broken line refer to bimodal samples. The numbers correspond to places listed in appendices 1 to 3 and table 1. ●—"East Cornish" populations; ▲—the south-western sub-area, including Lundy Island; ■—the remainder of southern England and its periphery.

English distribution since 1956, and that populations in the south-eastern sub-area showed slight differences in 1956, though not in 1957, from those of the more northerly counties.

In figs. 1 and 2, the south-western samples are represented by triangles to distinguish them from the rest of the former South English stabilisation area. The numbers following the places mentioned below refer to entries in these figs.

1956, *O.E. populations* included Middleton East (10) and, marginally, Woolbury Ring (27) in Hampshire, Sherborne (21) in Dorset and Newton Abbot (13) in Devon.

1956, *N.E. populations* included Middleton West (11), Worthy Down (28), Canterbury (1) and Shoreham (22) in the south-east sub-area; these four maintained modes at 0 spots between 50 per cent. and 60 per cent. and reference to fig. 1 will show that they are somewhat distinct from the samples obtained in the more northerly sub-area, which includes Chelmsford (3), Holt (6), Ipswich (7), Rugby (19), Cothill (4) and perhaps the small sample from Oxford,

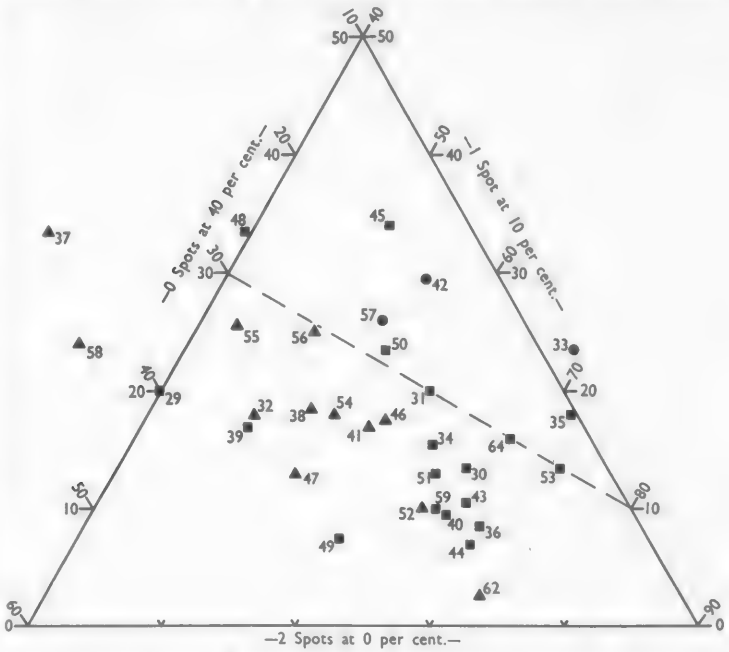


FIG. 2.—*Maniola jurtina*: English mainland and peripheral populations—female spot-distributions 1957. For explanation see fig. 1 on page 366.

Parks (17). The south-western sub-area was strongly stabilised, as reference to fig. 1 will show.

1956, *P.C. populations* included Oxford, Medley Manor (16), Tiverton (25), Taunton (23) and perhaps Newbury (12), where our sample was very small.

1957, *O.E. populations* included all localities in the south-eastern and northerly sub-areas, though Ipswich (39 and 40), Oxford, Medley Manor (48 and 49) and Oxford, Parks (50 and 51) only showed this distribution in the second phases of their emergences. One exceptional south-western locality, Pilistreet (52) near the border of the East Cornish stabilisation, also showed the O.E. distribution (see p. 380).

1957, *N.E. populations* included all south-western localities except Pilistreet (52); also the first phase of the Ipswich emergence (39).

The two outstanding western populations Newton Abbot (47) and Sherborne (54) moved over from O.E. to N.E. against the general trend.

1957, *P.C. populations* included Newbury (45) and the first phases of the two Oxford samples (48 and 50). Hoylake (35), with a large

TABLE 1

*Spot-distributions of* M. jurtina *populations in areas peripheral to the former South English stabilisation area*

| Locality | Year | | Spots | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 | 5 | |
| — Lundy Island . | 1955 | ♂ | 5 | 5 | 34 | 2 | ... | ... | 46 |
| | | ♀ | 47 | 18 | 5 | ... | ... | ... | 70 |
| 61. „ „ . | 1956 | ♂ | 3 | 7 | 62 | 24 | 1 | ... | 97 |
| | | ♀ | 45 | 21 | 14 | 4 | ... | ... | 84 |
| 62. „ „ . | 1957 | ♂ | 1 | 1 | 14 | 3 | ... | ... | 19 |
| | | ♀ | 29 | 10 | 1 | ... | ... | ... | 40 |
| 63. Isle of Man . | 1956 | ♂ | 7 | 22 | 85 | 13 | ... | 1 | 128 |
| | | ♀ | 101 | 15 | 2 | ... | ... | ... | 119 |
| 64. Pont-l'Evêque, *Normandy* | 1957 | ♂ | 1 | 1 | 27 | 10 | ... | ... | 39 |
| | | ♀ | 47 | 11 | 11 | 3 | ... | ... | 72 |
| 65. Sutherland . | 1956 | ♂ | 4 | 27 | 101 | 19 | 6 | ... | 161 |
| | | ♀ | 73 | 26 | 6 | 2 | ... | ... | 107 |
| 66. Sutherland (area "D") . | 1956 | ♂ | 2 | 18 | 33 | 2 | ... | ... | 55 |
| | | ♀ | 10 | 5 | ... | ... | ... | ... | 15 |

The serial numbers in the left-hand column refer to the corresponding entry in figs. 1 and 2.

mode at 0 and a small, possibly not significant, mode at 2 spots, seems aberrant.

### (iv) *Long-term behaviour of stabilisations*

It should not be thought that the events described in Southern England are unique. Although so far as our information goes the East Cornish and West Cornish (Penwith) stabilisations seem to have remained unaltered from 1952 to 1958, the fourth stabilisation known to us, that of the large islands of Scilly, after being in force from at least 1950 to 1955, began to break up in the direction of lower spotting in 1956 and had been partly restored by 1958. As in South England, one area (the largest of the islands), remained undisturbed throughout. Details of these events will be communicated in a paper devoted to the Isles of Scilly ; an account of the large island stabilisation up to 1955 is given in Dowdeswell, Ford and McWhirter (1957).

### (v) *Populations in areas peripheral to the former South English stabilisation area*

We have information on the condition of *M. jurtina* in the following areas :—Lundy, 1955-57 ; Isle of Man, 1956 ; Sutherland, Scotland, 1956 ; Pont-l'Evêque, Normandy, 1957. The data are listed in table 1.

The Sutherland samples are fully discussed in Forman, Ford and McWhirter (1959). It will be noticed that the Lundy Island population tends to conform not with the south-western region (Devon, Somerset and Dorset), as might be expected from its geographical position, but with the more remote parts of Southern England. The Manx sample maintains its strong deviation towards very low spot-values in both sexes which has been observed since 1951. Our first sample from Normandy seems to conform generally to the South English stabilization.

## 2. THE WEST DEVON AND CORNWALL AREA

### (i) *Survey of previous results* *

By the end of the 1951 season it had become clear that spotting in the females of *Maniola jurtina* was stabilised throughout the South of England with frequencies descending from a single mode at 0 spots. This pattern of frequency-distribution will be referred to as the "Southern English stabilisation". As already explained, however, minor variants can be recognised within it (the Old English and the New English forms, with more and less pronounced modes respectively). This general type of unimodal spotting distinguished all the populations studied from the North Sea to east Devon, though they are subject to great environmental differences, of climate, soil and vegetation.

As a result of work in previous years, it had already been realised that, widespread as it is, the Southern English stabilisation breaks down to other and characteristic values in some parts at least of Cornwall (Falmouth in the south and Hayle in the north). It was therefore decided to explore the situation from east Devon westwards in 1952.

Collecting was extremely hampered by wet weather, following long-continued heat and drought which had brought out the species unusually early, before we ourselves could be in the field. Five samples from central and west Devon were obtained. Three were caught in the middle of the county, respectively at Okehampton, Lydford and Holsworthy. The others were taken near t' south coast, at Newton Abbot (mid-Devon) and at Noss Mayo (five miles east of Plymouth). In all of them, the females were unimodal at 0 spots as in the rest of England eastwards, and the five populations

* The individual data upon which the results referred to in this sub-section are based can be obtained from Dowdeswell and Ford, 1953.

were homogeneous with one another ($\chi^2_{(4)} = 5{\cdot}39$, with $P$ between 0·3 and 0·2).

It should here be mentioned that in 1951 an additional sample (not published in our 1953 paper) was obtained only a mile or so from Plymouth, though still to the east, in the Plymstock area (see table 2). The females are very homogeneous when compared with the samples obtained the following year in the above five Devon localities ($\chi^2_{(2)} = 0{\cdot}362$, with $P = 0{\cdot}9$ to 0·8). It will be noticed that the high mode at 0 (over 60 per cent.) is consistent with the Old English type. This is confirmed by the males, which have a large mode at 2 spots and, for what the figures are worth, approximately equal numbers above and below that value.

TABLE 2

*Spot-distributions at Plymstock, 1951*

| | Spots | | | | | Total |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | |
| Females . | 34 | 14 | 4 | 2 | ... | 54 |
| Males . . | 1 | 3 | 19 | 2 | 1 | 26 |

Proceeding farther west, to the neighbourhood of the Devon-Cornwall border and east Cornwall, samples from another group of five localities (Plymouth North, Plymouth Roborough, Tavistock, Lewannick and Lanivet) were obtained in 1952. Here we pass to a situation of a different kind, in which the females are bimodal with a greater mode at 0 spots and a lesser at 2. These five populations also proved homogeneous with one another ($\chi^2_{(12)} = 8{\cdot}778$, with $P = 0{\cdot}8$-$0{\cdot}7$), in spite of considerable differences in habitat: coastal, inland and a locality (Lanivet) separated from the rest by the granite intrusion of Bodmin Moor. The difference between this group and the one just referred to from Devon is immensely significant ($\chi^2_{(3)} = 41{\cdot}481$). When the 1951 Plymstock sample is included, the distinction is increased to $\chi^2_{(3)} = 50{\cdot}978$.

After the 1952 season we were, then, faced with the following situation. A stability, characterised by female spotting unimodal at 0 spots, persisted from the coast of the North Sea across Southern England to west Devon. Near the Cornish border, and for a considerable distance beyond, this is replaced by a bimodal spot-distribution, also homogeneous. That area was sampled well inland (Lewannick), half-way thence to the south coast (Tavistock), at two places near Plymouth, and thirty miles westwards (Lanivet).

Although we are not in this account concerned with the situation in west Cornwall, it is desirable for completeness to mention that *Maniola jurtina* becomes rare or absent in the country immediately

east of the Hayle-Marazion isthmus, isolating an entirely distinct and apparently stabilised type on the Land's End peninsula ; one in which the females are unimodal at 2 spots like the males, only with the mode less pronounced. Two somewhat exceptional populations are left on either side of this stretch of isolating territory ; in the Falmouth district to the south and on the Hayle sandhills, in wholly abnormal conditions, to the north.

The whole situation here described is the one which Dobzhansky and Pavlovsky (1957) summarise in the following terms : " Populations of the butterfly *Maniola jurtina* are rather uniform throughout southern England, despite some obvious environmental diversity in different parts of this territory." In reality, the respective stabilisations persisted in the face of great environmental differences, while the chances (measured by $\chi^2_{(3)} = 41{\cdot}481$ with $P = 5{\cdot}17 \times 10^{-9}$) * are almost 1 in 200 million that the distinction between the Devon (*i.e.* Southern English) and East Cornish stabilisations is fortuitous. The difference is even greater when the Plymstock sample, not previously described, is included (p. 370). Considering this, and the other facts mentioned here, it seems that Dobzhansky and Pavlovsky's statement has not been based upon a sufficiently detailed examination of the published data.

### (ii) *Results obtained in* 1956 †

Subsequently to 1952, work on *Maniola jurtina* in the Isles of Scilly prevented us for some years from returning to the study of this insect on the mainland. In 1956, however, we determined to examine the way in which the spot-distributions found respectively in Devon and east Cornwall change from one to the other.

As explained previously, we already knew that the difference between them is very great and that each is homogeneous over a wide and highly diversified area, coastal and inland. It was also clear that there was no tendency for the two types to approximate even in the localities we had examined where they approached most closely. In the inland sector, which was the one we selected for study (see map, fig. 3), these were twelve miles apart, at Lydford (Devon) and Lewannick (Cornwall). Provided that the diversity had persisted throughout the intervening four years, the problem was likely to be of much interest since it will be seen that at its longest the cline involved could be no more than twelve miles. Yet the facts prove more surprising than anything we had anticipated.

The work was conducted by K. G. McWhirter and E. B. Ford without W. H. Dowdeswell who, unfortunately, could not be present in 1956. Our first task was to determine whether the Devon and East Cornwall populations previously studied still remained dissimilar. We therefore visited the large field (SX634992) which had provided the

* This extension of the $\chi^2$ table was kindly calculated for us by Mr N. T. J. Bailey.

† A combination of letters and numbers in brackets following a locality represents its position on the National Grid.

Okehampton sample in 1952 ; also the Lydford locality (SX487863) twelve miles farther west-south-west. The results are shown in tables 3 and 4 for the two sexes separately, where they are compared with the

TABLE 3

*The Devon and Cornish spot-distributions for 1952 and 1956 compared in the Okehampton to Lewannick transit ; females*

| Locality | Year | Spots | | | | | | Totals |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | |
| Okehampton, *Devon* . | 1952 | 36 | 9 | 5 | 3 | ... | ... | 53 |
| ,, ,, . | 1956 | 45 | 29 | 20 | 5 | ... | ... | 99 |
| Lydford, *Devon* . . | 1952 | 21 | 12 | 3 | ... | ... | ... | 36 |
| ,, ,, . . | 1956 | 50 | 27 | 20 | 11 | ... | ... | 108 |
| Lewannick, *Cornwall* . | 1952 | 28 | 17 | 21 | 7 | ... | ... | 73 |
| ,, , . | 1956 | 44 | 18 | 30 | 10 | ▪ | ... | 104 |

1952 data when available. It is evident that the females are still clearly unimodal at 0. This mode is, however, somewhat less accentuated, and to a significant degree. The difference between the years being

TABLE 4

*The Devon and Cornish spot-distributions for 1956 (compared with 1952 where available) ; males*

| Locality | Year | Spots | | | | | | Totals |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | |
| Okehampton, *Devon* . | 1952 | ... | ... | 14 | 6 | 2 | ... | 22 |
| ,, ,, . | 1956 | 1 | 8 | 55 | 18 | ▪ | ... | 83 |
| Lydford, *Devon* . . | 1956 | 4 | 8 | 49 | 15 | 5 | 2 | 83 |
| Lewannick, *Cornwall* . | 1952 | 2 | 2 | 25 | 4 | 2 | ... | 35 |
| ,, ,, . | 1956 | ▪ | ▯ | 58 | 27 | ▯ | 1 | 104 |

measured by $\chi^2_{(2)} = 7{\cdot}004$ at Okehampton, and by $\chi^2_{(2)} = 6{\cdot}228$ at Lydford, both giving $P$ between 0·05 and 0·02. This indicates a shift from the Old English to the New English types within the typical Southern English female stabilisation : an adjustment which by 1956 had occurred widely in the rest of Southern England eastwards. The females of the Okehampton and Lydford populations for that year are evidently homogeneous (and see p. 375) when compared with one another, as they are when compared jointly with the nine " New

English" spot-distributions found east of Devon in the same season * ($\chi^2_{(3)} = 1{\cdot}14$, with $P$ from 0·8 to 0·7).

In these nine "New English" populations, the females are extremely homogeneous with one another ($\chi^2_{(16)} = 11{\cdot}144$). On the other hand, the males are heterogeneous ($\chi^2_{(16)} = 30{\cdot}56$) being, as usual in that sex, more prone to be independently adapted to the minor environmental distinctions of each locality. The male spot-distributions at Okehampton and Lydford (table 4) are, however, evidently homogeneous in 1956.

We next proceeded to reinvestigate the nearest district previously studied within the East Cornish stabilisation: that is to say, Lewannick. Here we found that we could no longer obtain samples in the two neighbouring localities where we collected in 1952. One was a large sloping meadow which had now been ploughed. The other was a narrow valley (SX292797) in which the grass had become much invaded by bracken and brambles from the steep sides, so destroying the greater part of the insect's habitat.

We accordingly searched the Lewannick neighbourhood for another area where the butterfly was common. This we found just over three-quarters of a mile eastwards, in the valley of the Inny near Drinnick where the river is crossed by Trekelland Bridge (SX301797). The sample obtained at this place showed a spot-distribution entirely distinct in character from that of the Okehampton and Lydford specimens, being of the East Cornish type (table 3); that is, bimodal in the female. In that sex it was closely homogeneous with the condition as established in the same district four years before ($\chi^2_{(3)} = 1{\cdot}092$, with $P = 0{\cdot}8$ to $0{\cdot}7$), but it was only doubtfully so in the males ($\chi^2_{(3)} = 3{\cdot}007$, with $P = 0{\cdot}1$ to $0{\cdot}05$).

Owing, as it proves, to the small size of the sample which it was possible to collect, it was in neither sex fully heterogeneous with the estimate for the combined Okehampton and Lydford populations, though it approached this in the females (for which the difference is measured by $\chi^2_{(3)} = 6{\cdot}867$, with $P = 0{\cdot}1$ to $0{\cdot}05$; while for the males, $\chi^2_{(3)} = 3{\cdot}575$, with $P$ nearly $0{\cdot}3$). It will be shown, however, that the "Lewannick" sample can be added to that from another East Cornish population with which it is homogeneous. The difference between the two combined, compared with the spot-distributions found in Devon, is heavily significant in the females though not in the males (p. 376).

We therefore set out to analyse the transition between the two types. This we did by sampling the *M. jurtina* population in the twelve miles which separate these localities. We decided first to subdivide the distance, which there includes the county boundary, into three blocks, working from north-east to south-west. We accordingly

* Shapwick (Somerset), Middleton West (Hampshire), Worthy Down (Hampshire), Shoreham (Sussex), Canterbury (Kent), Cothill (Berkshire), Rugby (Warwickshire), Ipswich (Suffolk), Holt (Norfolk).

advanced approximately four miles from Lydford finding, with some difficulty, a suitable collecting ground in a valley at Chillaton (SX435818). Here, to our surprise, the population proved completely Southern English in both sexes (tables 5 and 6).

TABLE 5

*Spot-distributions in the localities sampled between the known Southern English (Lydford) and East Cornish (Lewannick) stabilisations in 1956; females*

| Locality | Spots | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | |
| Chillaton . . . . | 38 | 18 | 13 | 6 | 3 | ... | 78 |
| Tamar West . . . . | 30 | 19 | 9 | 5 | 1 | 1 | 65 |
| East Larrick . . . . | 20 | 11 | 6 | 1 | ... | ... | 38 |
| West Larrick . . . | 16 | 6 | 13 | 5 | 1 | ... | 41 |

We next proceeded a further four and a half miles in the direction of the Lewannick area and discovered a locality near Hexworthy, in the Tamar valley on the west side of the river (SX366811), where the butterfly was in some numbers. It was, however, not easily caught,

TABLE 6

*Spot-distributions in the localities sampled between the known Southern English (Lydford) and East Cornish (Lewannick) stabilisations in 1956; males*

| Locality | Spots | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | |
| Chillaton . . . . | ... | 9 | 63 | 17 | 9 | ... | 98 |
| Tamar West . . . . | 1 | 7 | 59 | 20 | 2 | ... | 91 |
| East Larrick . . . . | ... | 6 | 51 | 22 | 3 | ... | 82 |
| West Larrick . . . | 1 | 1 | 35 | 10 | 6 | 1 | 59 |

as the insects ranged widely at a rather low density over extensive fields. The resulting sample (tables 5 and 6) proved completely Southern English in spotting, without any indication that we were now within four miles of the "Lewannick" locality at the Inny Bridge which, as we knew, falls within the East Cornish stabilisation.

The intervening country, though irregular, on the whole rises from the valley of the Tamar until within a quarter of a mile of the Inny it drops steeply down to that river. We decided to advance approximately half-way across this area to Larrick, where we found a suitable collecting ground in the valley of a stream which runs eastward from the heights above the Inny valley. This stream is (at

SX329806) crossed by the lane from Launceston to Trebullett. The position is approximately two and a quarter miles from the population (proved Southern English) on the west side of the Tamar valley, and one and three-quarter miles from that, proved East Cornish, on the banks of the Inny. From here we moved westwards collecting on the southern side of the stream, keeping an account of our results on the way.

By the time we had worked through two fields before crossing a lane at Larrick farm and three subsequently, we had accumulated 82 males and 38 females. We accordingly paused on the east side of the hedge between the third and fourth fields from the farm (SX322803)

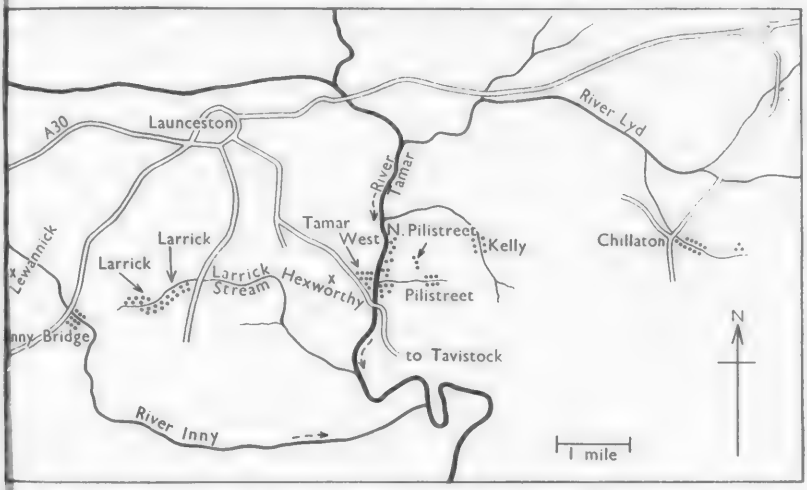


FIG. 3.—Map of the region in which we studied the transition from the Devon to the Cornish types of spotting in *Maniola jurtina*. The localities where the samples were obtained are indicated by dots. (The river Tamar is here the county boundary.)

and scored our sample (tables 5 and 6). Since we were making our way up the course of the stream, which ran beside us on our right, we describe this area as "East Larrick" (SX322 to 328 and 803 to 807). The result was still completely Southern English, though we were now no more than a mile and a quarter from the East Cornish population by the Inny Bridge (Lewannick). Indeed the spot-distributions found in the whole series of localities from Okehampton to this point (Okehampton, Lydford, Chillaton, Tamar West and East Larrick) are extraordinarily homogeneous. Their uniformity is measured by $\chi^2_{(8)} = 2{\cdot}741$ giving $P = 0{\cdot}95$ to $0{\cdot}9$ for the females, and by $\chi^2_{(8)} = 3{\cdot}662$ giving $P = 0{\cdot}9$ to $0{\cdot}8$ for the males.

We now climbed through the hedge and proceeded across the fourth field westwards from Larrick farm. It is of large size and the greater part is flat, but it slopes steeply down to the stream on its

northern side. As we advanced, making a rough assessment of our captures on the way, we became aware that a change in spot-distribution had at last occurred. We proceeded to collect in the next field westwards and on some rough ground, interspersed with gorse and bramble, separated from it by a narrow marshy copse. This marsh continued the line of the stream which had, in fact, turned northwards just previously.

These two fields, and the piece of rough ground beyond them, we describe as the "West Larrick" locality (SX318322 and SX801803). Having accumulated 59 males and 41 females there (tables 5 and 6), we sat down to score them. The result was spectacular, confirming the counts made while collecting. The females were completely East Cornish in type, bimodal and highly homogeneous with the Lewannick sample by the river Inny ($\chi^2_{(8)} = 0{\cdot}512$, with $P = 0{\cdot}95$-$0{\cdot}90$). They are heterogeneous when compared with those from East Larrick in the fields immediately to the east ($\chi^2_{(2)} = 7{\cdot}350$, with $P = 0{\cdot}05$-$0{\cdot}02$).

When the females of the East Cornish stabilisation from West Larrick and the Lewannick area (at the Inny Bridge) are combined, which their extreme homogeneity fully allows, they may be compared with the Southern English series from Okehampton to East Larrick, itself already shown remarkably uniform. The result further demonstrates the reality of the difference between these two types of spot-distribution, for the heterogeneity between them is measured by $\chi^2_{(3)} = 14{\cdot}539$, giving $P = 0{\cdot}01$-$0{\cdot}001$.

The males, on the other hand, are moderately homogeneous with the Southern English type at East Larrick ($\chi^2_{(3)} = 5{\cdot}550$, with $P = 0{\cdot}2$-$0{\cdot}1$). They are fully so with those of the Lewannick (Inny Bridge) area ($\chi^2_{(3)} = 2{\cdot}008$, with $P = 0{\cdot}7$-$0{\cdot}5$). The males from West Larrick and Lewannick combined do not differ significantly from the Southern English series from Okehampton to East Larrick ($\chi^2_{(3)} = 5{\cdot}206$, with $P = 0{\cdot}2$-$0{\cdot}1$). As already explained, they do not therefore provide a clear criterion of the distinction between the East Cornish and main Southern English stabilisations.

When the female samples along the transect are compared, it is found that the difference between the Southern English and the East Cornish spot-distributions is greatest at the boundary between them. This is most conveniently shown when the average spot-numbers are considered. These are, of course, greater in the Cornish stabilisation, with its subsidiary mode at 2, than in the Southern English, with its single mode at 0. The values may be plotted against the distance along the transect from a given datum point, in this case Lewannick (see fig. 4). It will then be seen that the spot-average for West Larrick is even higher than that for Lewannick, while that for East Larrick is even lower than that for Tamar West. This is the precise opposite of the situation found in a cline. No comparable effect can be detected when the male spot-values are plotted in a similar manner.

The evidence consequently indicates that the Southern English and East Cornish spot-distributions change abruptly from one to the other. This they do within a few yards ; a transition which, in 1956, occurred at the ordinary field-hedge between the West and East Larrick collecting areas (SX322803). Yet this presents no barrier to the insects, which are constantly seen crossing it.

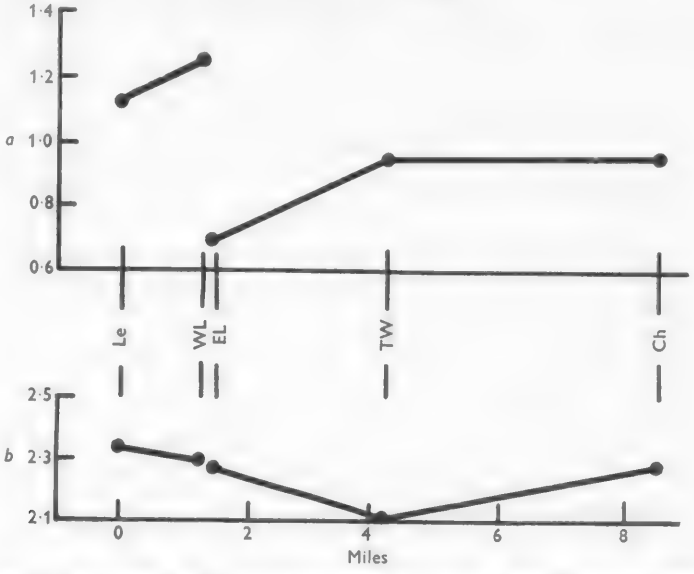


FIG. 4.—*Maniola jurtina* : the boundary between the East Cornish and Southern English stabilisations—1956. The average spot-numbers of (*a*) the females and (*b*) the males are plotted against the distance along the transect. For explanation see text page 376. (Le, Lewannick ; WL, West Larrick ; EL, East Larrick ; TW, Tamar West ; Ch, Chillaton.)

### (iii) *Results obtained in* 1957

It was important to determine whether the abrupt discontinuity in spotting between the Southern English and East Cornish stabilisations, discovered in 1956, persisted the following year. We therefore again studied the spot-distributions of *Maniola jurtina* in the critical area of the Devon-Cornwall border in 1957. The work was more difficult than in the previous season because the butterfly was on the whole considerably rarer, due, no doubt, to the abnormal weather conditions of the spring and early summer. An unprecedented period of drought had continued up to early July and was followed by much rain for the rest of that month.

W. H. Dowdeswell, who collaborated in initiating these studies, and has great experience of them, accompanied us this year. We were joined also by E. R. Creed.

Our first objective was to collect in the Lewannick area at Inny Bridge, where we captured 126 males and 155 females (see tables 7 and 8). The two sexes were respectively homogeneous when compared with the samples obtained the previous year, the females being

TABLE 7

*Spot-distributions in the localities sampled in the 8¼ miles from Chillaton (Devon) to Lewannick (Cornwall) in 1957 ; females. The localities are arranged from east to west*

| Locality | Spots | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | |
| Chillaton | 27 | 19 | 10 | 5 | ... | ... | 61 |
| Kelly | 50 | 23 | 15 | 10 | 2 | ... | 100 |
| Pilistreet | 51 | 20 | II | 6 | ... | ... | 85 |
| N. Pilistreet | 26 | 11 | II | 4 | 1 | ... | 50 |
| Tamar West | 84 | 32 | 41 | 12 | ... | ... | 169 |
| East Larrick | 27 | 3 | 9 | 5 | 1 | ... | 45 |
| West Larrick | 1 | 2 | ... | ... | 1 | ... | 4 |
| Lewannick | 80 | 23 | 43 | II | 1 | ... | 155 |

TABLE 8

*Spot-distributions in the localities sampled in the 8¼ miles from Chillaton (Devon) to Lewannick (Cornwall) in 1957 ; males. The localities are arranged from east to west*

| Locality | Spots | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | |
| Chillaton | ... | 8 | 42 | 15 | 4 | 1 | 70 |
| Kelly | 1 | 5 | 22 | 11 | 2 | 1 | 42 |
| Pilistreet | ... | ... | 25 | 12 | 2 | ... | 39 |
| N. Pilistreet | ... | 3 | 25 | 9 | 3 | ... | 40 |
| Tamar West | 2 | 4 | 35 | 9 | 4 | 2 | 56 |
| East Larrick | 1 | 2 | 17 | 7 | 4 | ... | 31 |
| West Larrick | ... | 1 | 3 | ... | ... | ... | 4 |
| Lewannick | 2 | 7 | 79 | 29 | 9 | ... | 126 |
| | | | | | | ... | |

bimodal (in that sex the comparison is measured by $\chi^2_{(3)} = 3{\cdot}912$, with $P = 0{\cdot}3$ to $0{\cdot}2$ ; while for the males $\chi^2_{(3)} = 1{\cdot}250$, with $P = 0{\cdot}8$ to $0{\cdot}7$). It was evident, therefore, that in 1957 the East Cornish type of spotting persisted unaltered in the Lewannick district.

We also collected on the west side of the river Tamar, near Hexworthy, in the locality studied in 1956 (p. 374). The butterflies were difficult to obtain, being widespread at a low density, so that it was necessary to spend two days there. However, we were at length

fortunate in discovering two fields in which considerable numbers were to be found in long grass. In all, we amassed 169 females and 56 males (see tables 7 and 8). Here we had been within the normal Southern English stabilisation the previous year, so we were surprised to find the females were now bimodal and completely of the East Cornish type.

Between the two localities so far studied (Inny Bridge and the west side of the Tamar near Hexworthy) lay the critical Larrick area which previously included the abrupt transition in spotting. Here *M. jurtina* was much rarer owing to the parched condition of the fields. The West Larrick locality was practically denuded of butterflies and a long search resulted only in 4 females and 4 males (see tables 7 and 8). The East Larrick region, being moister, was less unfavourable and there we amassed 45 females and 31 males (see tables 7 and 8) : a considerable number of these were caught in an orchard separated from the first field west of Larrick farm only by a stream narrow enough to jump over and a straggling hedge. It will be remembered that here in the previous year the females were of the normal Southern English type, being unimodal at 0 spots. They were now bimodal, the samples for 1956 and 1957 being heterogeneous ($\chi^2_{(2)} = 7{\cdot}988$, with $P < {\cdot}02$). This result accords with the similar situation two and a half miles farther east on the Cornish bank of the Tamar, suggesting that we had to deal with a change affecting a tract of country rather than independent adjustments to individual localities.

The spot-distributions in these three places, Lewannick, East Larrick and the west side of the Tamar, proved to be homogeneous (for the females, $\chi^2_{(6)} = 7{\cdot}925$, with $P = 0{\cdot}3$ to $0{\cdot}2$ ; for the males, $\chi^2_{(6)} = 3{\cdot}036$, with $P$ approximately $0{\cdot}8$). The 4 females and 4 males captured at West Larrick in 1957 are omitted from this calculation. Clearly it would not be right to include with East Larrick a sample too small to be judged independently, one from a population which had in fact been heterogeneous with it the previous year.

Thus the Cornish form of spotting evidently now affected the *jurtina* population as far east as the Tamar. It was necessary, therefore, at this stage to locate an unchanged English stabilisation as a preliminary to discovering whether or not the transition between the two types remained abrupt. Consequently we advanced four and a half miles to the next locality east of the Tamar where we had obtained information the previous year : that is to say, Chillaton. Here the Southern English stabilisation still persisted in 1957 (see table 7), the females being unimodal at 0 spots.

It was now clear that in the transect which we were studying, the change from the East Cornish to the Southern English spotting occurred somewhere between Chillaton and the Tamar ; presupposing, that is to say, that the distinctive types were to be found uniformly up to some transition area, broad or narrow, rather than in an independent scatter of localities. Consequently we looked for a suitable collecting

ground in this stretch of country. We found one in a valley at Kelly (SX388813) approximately two and three-quarter miles west of Chillaton. Here the *jurtina* population was still of the Southern English type, unimodal at 0 spots (see table 7), though we were now only one and a quarter miles east of the Tamar near Hexworthy.

At that point the river flows close to the eastern side of its flood-plain. A single row of fields about 150 yards wide separates it from a steep wooded bank, about 150 feet high, above which are open fields. We collected from the edge of these woods, at the top of the declivity, back for a few hundred yards, choosing the north side of a small valley near Pilistreet (SX375807). The sample obtained there was again completely Southern English, the females being unimodal at 0 spots. So too, was another (which we describe as North Pilistreet), running

TABLE 9

*Spot-distributions of an intermediate type in the flood-plain, about 160 yards wide, east of the Tamar between the river and its escarpment*

| | Spots | | | | | Total |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | |
| Females . . | 67 | 36 | 34 | 5 | ... | 142 |
| Males . . | ... | 2 | 23 | 11 | 5 | 41 |

northwards for about 300 yards along the upper edge of the woods and separated from the last locality by about 150 yards (see table 7).

We had, therefore, explored a transect running from the edge of the Tamar Valley eastwards to Chillaton, approximately four miles away. The four populations included in it proved to be homogeneous in both sexes (for the females $\chi^2_{(9)} = 6{\cdot}239$, with $P > 0{\cdot}7$; for the males, $\chi^2_{(3)} = 0{\cdot}935$, with $P > 0{\cdot}8$).

We had now established that the Cornish and Southern English populations extended respectively west and east from the Tamar, each being homogeneous. The distinction between the bimodal Cornish and the unimodal English type of female is heavily significant, with $\chi^2_{(3)} = 18{\cdot}086$, giving $P < 0{\cdot}001$. The males as before, do not discriminate between the two stabilisations ($\chi^2_{(3)} = 1{\cdot}182$, with $P$ between 0·7 and 0·5).

In addition to the river itself (about 15 yards broad) and the precipitous woods from which the butterfly is absent, the English population at Pilistreet and the Cornish on the west bank of the Tamar are separated only by the eastern flood-plain, here no more than 150 yards wide, in which the butterfly is established and in which with difficulty we amassed a considerable sample (see table 9). This proved to be significantly intermediate in type, with nearly equal values at one and two spots in the female. In that sex, the differences involved

are measured as follows : when compared with the Cornish form, from Lewannick to the west bank of the Tamar, $\chi^2_{(3)} = 8{\cdot}022$ (with $P = 0{\cdot}05$-$0{\cdot}02$), while compared with the Southern English form, from Chillaton to North Pilistreet, $\chi^2_{(3)} = 10{\cdot}663$ (with $P < 0{\cdot}02$).

We attempted two further collections in what might be intermediate areas. One of these (A, see table 10) consisted of two contiguous fields, carved out of the hanging woods which here isolate the eastern flood-plain from the Pilistreet locality. At the bottom, one of them is separated only by a line of trees from the plain itself. We could only obtain a few butterflies here. These were probably of the intermediate type, but the numbers are too small to be decisive. The other sample (B, see table 10) was caught in a steeply sloping field running south

TABLE 10

(*See text*)

| Localities | Spots | | | | | Total |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | |
| A (males) . | ... | ... | 5 | 4 | ... | 9 |
| (females) . | 3 | 5 | 4 | 1 | ... | 13 |
| B (males) . | ... | 3 | 14 | 11 | 1 | 29 |
| (females) . | 14 | 8 | 5 | 2 | ... | 29 |

along the top of the woods from the Pilistreet area. Its spot-distribution appears to be typical Southern English, as is to be expected here, but the numbers seem hardly large enough to warrant inclusion in the general calculations.

When a comparison is made of the average spot-numbers for the samples along the transect, in a similar manner to that for the 1956 results (p. 376), an effect representing the reverse of a cline is again obtained in the females (see fig. 5). However, this time the divergence from the normal is confined to the English side of the boundary (the Pilistreet, though not the North Pilistreet sample). It should be pointed out that, owing to the higher mode at 0 spots, the average spot-numbers of the East Cornish samples are rather lower than in 1956.

In the males the condition in 1957 differs not only from that in the females but also from that in the males the previous year. In 1956 their spot-averages did not vary at the boundary. In 1957 (see fig. 5) the male samples from the English population at Pilistreet and from the intermediate one at Tamar East, have higher spot-averages than the other English or East Cornish samples, whereas the females from Pilistreet have a considerably lower spot-average than is obtained either to the east or west.

Powerful selection-pressures are no doubt operating universally

on *Maniola jurtina*. The reverse cline effect observed at the junction between the East Cornish and Southern English stabilisations in both 1956 and 1957 suggests that here they are of quite exceptional force.

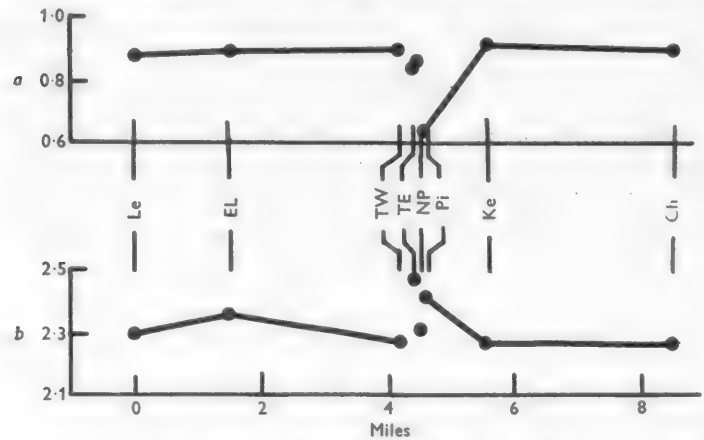


FIG. 5.—*Maniola jurtina* : the boundary between the East Cornish and Southern English stabilisations—1957. The average spot-numbers of (*a*) the females and (*b*) the males are plotted against the distance along the transect. For explanation see text pages 376 and 381. (Le, Lewannick ; EL, East Larrick ; TW, Tamar West ; TE, Tamar East ; NP, North Pilistreet ; Pi, Pilistreet ; Ke, Kelly ; Ch, Chillaton.)

## 3. DISCUSSION

The most striking of the facts communicated above is the sudden change from the South English (N.E.) to the East Cornish (E.C.) stabilisation (pp. 371-82). Before we undertook the task of transecting the boundary between these two stabilisation areas we thought we might find, in the following order of likelihoods, (i) a steep cline, or (ii) isolation, *i.e.* a region in which *M. jurtina* was rare or absent, or (iii) a confused pattern, in which populations with various or intermediate spot-frequencies could be found. It was, therefore, very surprising to find firstly, that the change occurred abruptly at a point where there was no barrier to *M. jurtina* and where the population was as dense as it was anywhere else on the transect, and secondly, that in the immediate neighbourhood of the boundary the female spot-distributions on each side actually diverged from one another, producing the effect which we describe as the precise opposite of a cline (p. 376).

Our findings in 1956 could be interpreted in two ways : (*a*) that the two populations were genetically isolated, or (*b*) that different norms of selection, acting on a genetically continuous population, were in force on either side of the boundary and that the geographic transition from one norm to the other was sharp. The discovery in

1957 that the clear-cut boundary between the two stabilisation areas had shifted some three miles eastward forced us to reject explanation (*a*) above and to accept (*b*). Clearly, in view of the life-history of *M. jurtina* no population could spread and replace another as rapidly and extensively as would be indicated by a shift of three miles per annum along this transect. Thus we visualise the South English-east Cornish boundary as one at which there is a change-over, not between genetically isolated populations, but between one set of selectional forces and another.

Turning to the data obtained in the former South English stabilisation area, the same kind of explanation offers itself irresistibly. We cannot easily imagine any process of population-replacement causing the change from O.E. to N.E. or P.C. spot-distributions, especially when two of these occur consecutively during the emergence at one locality. It is clear that populations of *M. jurtina* are subject to powerful, yet buffered, selection-pressures which are occasionally liable to change.

We have no hesitation in postulating strong selection-pressures. Let us consider two closely studied polymorphisms: Allison (1954) finds that the fitness of sickle-cell heterozygotes in Man is 1·26 times that of the normal homozygote in regions of East Africa where malaria is hyperendemic; Kettlewell (1958), discussing the spread of melanism in *Biston betularia* (L.) since the Industrial Revolution, postulates selective advantages in favour of the *carbonaria* gene rising to around 30 per cent. The spot-phenotypes of *M. jurtina* seem likely to be controlled by a polygenic system, but strong selection can, of course, still operate on characters governed simultaneously by several genes.

It is tempting to try to identify the selectional forces involved, in the case of *M. jurtina*, although these are immaterial to our thesis if it is admitted that spot-phenotypes are entirely or mainly genetically determined. Conclusive proof can only be obtained from individual breeding experiments, which, despite the great technical difficulties of controlled mating in the *Satyridæ*, we hope to perform. Existing data on a population basis, however, indicate that tendencies towards high or low spotting can be inherited from one year to another and we shall present a separate analysis dealing with this point.

In the Isles of Scilly we have noticed some degree of correlation between high spot-values and relative luxuriance of the habitat (McWhirter, 1957) and it was an obvious approach to an explanation of the sudden outbreak of high-spotting all over Southern England to examine whether this could be explained by the decimation of the rabbit population after the rapid spread of the virus disease, myxomatosis; in many areas this produced a lush growth of grasses, including those which are food-plants of *M. jurtina*. Such an hypothesis, however, can hardly be maintained. It is known that rabbits have been absent from Oxford, Medley Manor, since 1947, when they were destroyed by high floods; they had had no opportunity to recover before myxomatosis appeared. Yet the *M. jurtina* population in the vicinity

has conformed to the general pattern (1956, P.C.; 1957, P.C. then O.E.).

There are three types of discontinuity of spot-distributions discussed in this paper; local (especially in the region of the extraordinarily sharp boundary between the South English and East Cornish stabilisation areas), inter-seasonal and intra-seasonal. All three of these show a kind of "quantum" effect which is reflected in triangular diagrams such as figs. 1 and 2 above and in fig. 2 in McWhirter (1957), where data from the Isles of Scilly are similarly treated. In all these diagrams "clumping" of the entries is strongly marked. The true explanation of these effects will, we hope, emerge from further investigations.

## 4. SUMMARY

1. Between 1953 and 1955 the well-established South English stabilisation area, in which all populations of *M. jurtina* had female spot-distributions with large modes at 0 spots, broke up.

2. Two new types of distribution have appeared in this area, involving higher levels of spotting in both males and females, and these are defined.

3. The wave of high spotting reached a peak in 1956 and receded in 1957.

4. The inception of a new stabilisation area confined to the south-western part (Devon, Somerset and Dorset) of the former South English stabilisation area is described.

5. Not only local and inter-seasonal, but also intra-seasonal shifts in spot-distribution are now known in several places.

6. In some cases high spotting seems to have spread through a population after being at first confined to the early part of the emergence.

7. In 1952 it had been shown that while female spotting is unimodal at 0 spots in all areas sampled from the North Sea to west Devon, it is bimodal (with a greater mode at 0 and a lesser at 2 spots) in east Cornwall. These two types were stabilised in spite of great environmental differences within the regions they inhabit and persisted to within twelve miles of one another.

8. They still remained distinct in west Devon and east Cornwall in 1956.

9. The country separating them was studied in that year. It was then found that one type was replaced by the other in a few yards, at a single field-hedge which was not a barrier to the insect's movement.

10. The two populations proved to be subject to a phenomenon which represents the reverse of a cline. That is to say, their differences in spotting became accentuated as the boundary between them was closely approached.

11. This general situation persisted in 1957 when the boundary

separating the West Devon and East Cornish forms had, in the transect we were studying, moved three miles eastwards, to the river Tamar. It was, however, slightly less sharp since an intermediate population inhabited the eastern flood-plain, here only about 150 yards wide.

12. An effect representing the reverse of a cline could again be detected in 1957, for the characteristics of the West Devon, though this season not of the East Cornish, form were accentuated at the point where the two approached most closely.

13. It is argued that the most likely explanation of the sharp boundary at the Cornish border and of the sudden large changes in the South English populations is that strong selective forces operate on characters connected with spotting; these forces differ locally and, sometimes, when a stabilisation area passes into a fluid state, they appear to differ temporally.

14. We have considered, but regard as inconclusive, the evidence that the spread of the virus disease, myxomatosis, which has decimated the rabbit population and encouraged lush growth of grasses in many parts of Southern England, might be responsible for the wave of high-spotting which reached a peak in 1956.

15. Data from areas round the periphery of the former South English stabilisation area (Lundy Island, Isle of Man, Sutherland and Normandy) are briefly considered.

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## APPENDIX 1

*Spot-distributions of* Maniola jurtina, *1956*

(The serial numbers in the left-hand column refer to the corresponding entries in fig. 1.)

| Locality | | Spots | | | | | | Total | Spot-av. |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | | |
| 1. Canterbury, *Kent* . | ♂ | 4 | 5 | 45 | 21 | ... | ... | 75 | 2·11 |
| | ♀ | 17 | 10 | 6 | 2 | ... | ... | 35 | 0·80 |
| 2. Chillaton, *Devon* . | ♂ | ... | 9 | 63 | 17 | 9 | ... | 98 | 2·27 |
| | ♀ | 38 | 18 | 13 | 6 | 3 | ... | 78 | 0·95 |
| 3. Chelmsford, *Essex* . | ♂ | 1 | 3 | 27 | 14 | 2 | ... | 47 | 2·28 |
| | ♀ | 7 | 8 | 5 | 3 | ... | ... | 23 | 1·17 |
| 4. Cothill, *Berkshire* . | ♂ | ... | 5 | 38 | 21 | 2 | ... | 66 | 2·30 |
| | ♀ | 14 | 10 | 7 | 2 | 1 | ... | 34 | 1·00 |
| 5. East Larrick, *Cornwall* | ♂ | ... | 6 | 51 | 22 | 3 | ... | 82 | 2·27 |
| | ♀ | 20 | 11 | 6 | 1 | ... | ... | 38 | 0·68 |
| 6. Holt, *Norfolk* . | ♂ | 3 | 10 | 105 | 41 | 9 | 1 | 169 | 2·27 |
| | ♀ | 27 | 19 | 13 | 3 | 1 | ... | 63 | 0·92 |
| 7. Ipswich, *Suffolk* . | ♂ | 1 | 2 | 65 | 38 | 8 | ... | 114 | 2·44 |
| | ♀ | 42 | 32 | 23 | 7 | 1 | ... | 105 | 0·98 |
| 8. Lewannick, *Cornwall* | ♂ | 2 | 8 | 58 | 27 | 8 | 1 | 104 | 2·33 |
| | ♀ | 44 | 18 | 30 | 10 | 2 | ... | 104 | 1·12 |
| 9. Lydford, *Devon* . | ♂ | 4 | 8 | 49 | 15 | 5 | 2 | 83 | 2·18 |
| | ♀ | 50 | 27 | 20 | 11 | ... | ... | 108 | 0·93 |
| 10. Middleton East, *Hampshire* . . | ♂ | 2 | 5 | 32 | 8 | ... | ... | 47 | 1·98 |
| | ♀ | 93 | 43 | 12 | ... | ... | ... | 148 | 0·45 |
| 11. Middleton West, *Hampshire* | ♂ | 4 | 9 | 79 | 18 | 7 | ... | 117 | 2·13 |
| | ♀ | 50 | 30 | 15 | 7 | ... | ... | 102 | 0·79 |
| 12. Newbury, *Berkshire*. | ♂ | 1 | 1 | 12 | 7 | 2 | ... | 23 | 2·35 |
| | ♀ | 5 | 2 | 4 | ... | ... | ... | 11 | 0·91 |
| 13. Newton Abbot, *Devon* | ♂ | 4 | 14 | 59 | 23 | 6 | ... | 106 | 2·12 |
| | ♀ | 60 | 23 | 15 | 2 | 1 | ... | 101 | 0·62 |
| 14. Noss Mayo, *Devon* . | ♂ | 2 | 8 | 87 | 37 | 8 | 3 | 145 | 2·34 |
| | ♀ | 31 | 21 | 12 | 1 | 1 | ... | 66 | 0·79 |

APPENDIX 1—*continued*

*Spot-distributions of* Maniola jurtina, *1956*

| Locality | | Spots | | | | | | Total | Spot-av. |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | ▮ | 4 | 5 | | |
| 15. Okehampton, *Devon* | ♂ | 1 | ▮ | 55 | 18 | 1 | ... | 83 | 2·12 |
| | ♀ | 45 | 29 | 20 | 5 | ... | ... | 99 | 0·85 |
| 16. Oxford, Medley Manor | ♂ | 1 | 30 | 120 | 69 | 30 | 2 | 252 | 2·41 |
| | ♀ | 44 | ▮▮ | 29 | 8 | 2 | ... | 105 | 1·07 |
| 17. Oxford, Parks | ♂ | 1 | 7 | 31 | 13 | ▮ | ... | 60 | 2·33 |
| | ♀ | 5 | 5 | 3 | ... | ... | ... | 13 | 0·85 |
| 18. Plymouth, Roborough, *Devon* | ♂ | 2 | ▮ | 10 | 7 | ... | ... | 21 | 2·05 |
| | ♀ | 20 | 1 | 6 | 5 | ... | ... | 32 | 0·88 |
| 19. Rugby, *Warwickshire* | ♂ | 3 | 19 | 70 | 30 | 2 | ▮ | 126 | 2·12 |
| | ♀ | 27 | 23 | 14 | ▮ | 2 | ... | 74 | 1·12 |
| 20. Shapwick, *Somerset* | ♂ | 1 | 2 | 35 | 12 | 3 | ... | 53 | 2·26 |
| | ♀ | 18 | 11 | 9 | 1 | ... | ... | 39 | 0·82 |
| 21. Sherborne, *Dorset* | ♂ | 5 | 11 | 82 | 29 | 5 | ... | 132 | 2·14 |
| | ♀ | 28 | 10 | 7 | 3 | ... | ... | 48 | 0·69 |
| 22. Shoreham, *Sussex* | ♂ | ... | ▮ | 60 | 17 | 1 | ... | 86 | 2·13 |
| | ♀ | 21 | 14 | 6 | 1 | 1 | ... | 43 | 0·77 |
| 23. Taunton, *Somerset* | ♂ | ... | 2 | 30 | 19 | ▮ | ... | 54 | 2·43 |
| | ♀ | 12 | 3 | 5 | 1 | ... | ... | 21 | 0·76 |
| 24. Tamar West, *Cornwall* | ♂ | 3 | 7 | 59 | 20 | ▮ | ... | 91 | 2·12 |
| | ♀ | 30 | 19 | 9 | 5 | 1 | 1 | 65 | 0·94 |
| 25. Tiverton, *Devon* | ♂ | ... | 7 | 57 | 38 | ▮ | ... | 108 | 2·40 |
| | ♀ | 47 | 18 | 23 | 8 | 1 | ... | 97 | 0·95 |
| 26. West Larrick, *Cornwall* | ♂ | 1 | 6 | 35 | 10 | 6 | 1 | 59 | 2·29 |
| | ♀ | 16 | 6 | 13 | 5 | 1 | ... | 41 | 1·24 |
| 27. Woolbury Ring, *Hampshire* | ♂ | ... | 3 | 42 | 13 | 3 | ... | 61 | 2·26 |
| | ♀ | 38 | 18 | 8 | 3 | 1 | ... | 68 | 0·69 |
| 28. Worthy Down, *Hampshire* | ♂ | 2 | 7 | 73 | 35 | 2 | 1 | 120 | 2·26 |
| | ♀ | 60 | 40 | 17 | 3 | 1 | ... | 121 | 0·72 |

## APPENDIX 2

*Spot-distributions of* Maniola jurtina, *1957*

(The serial numbers in the left-hand column refer to the corresponding entries in fig. 2.)

| Locality | | Spots | | | | | | Total | Spot-av. |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | | |
| *29. Birdlip, *Gloucestershire* (to 21/vii) | ♂ | ... | 4 | 25 | 8 | 5 | ... | 42 | 2·33 |
| | ♀ | 6 | 6 | 3 | ... | ... | ... | 15 | 0·80 |
| 30. Birdlip, *Gloucestershire* (28/vii) | ♂ | ... | ... | 7 | 1 | 1 | ... | 9 | 2·33 |
| | ♀ | 29 | 9 | 6 | 1 | ... | ... | 45 | 0·53 |
| 31. Canterbury, *Kent* . | ♂ | ... | 1 | 23 | 3 | ... | ... | 27 | 2·07 |
| | ♀ | 12 | 4 | 4 | ... | ... | ... | 20 | 0·60 |
| 32. Chillaton, *Devon* . | ♂ | ... | 8 | 42 | 15 | 4 | 1 | 70 | 2·26 |
| | ♀ | 27 | 19 | 10 | 5 | ... | ... | 61 | 0·89 |
| 33. East Larrick, *Cornwall* | ♂ | 1 | 2 | 17 | 7 | 4 | ... | 31 | 2·35 |
| | ♀ | 27 | 3 | 9 | 5 | 1 | ... | 45 | 0·89 |
| 34. Heswall, *Cheshire* . | ♂ | 3 | 12 | 47 | 10 | 4 | ... | 76 | 2·00 |
| | ♀ | 28 | 10 | 7 | 2 | ... | ... | 47 | 0·64 |
| 35. Hoylake, *Cheshire* . | ♂ | 2 | 5 | 22 | 4 | 1 | ... | 34 | 1·91 |
| | ♀ | 20 | 3 | 5 | ... | ... | ... | 28 | 0·46 |
| 36. Holt, *Norfolk* . | ♂ | 4 | 12 | 128 | 39 | 9 | ... | 192 | 2·19 |
| | ♀ | 66 | 21 | 8 | 1 | ... | 1 | 96 | 0·47 |
| 37. Intermediate "A", *Devon*, (p. 381) | ♂ | ... | ... | 5 | 4 | ... | ... | 9 | 2·44 |
| | ♀ | 3 | 5 | 4 | 1 | ... | ... | 13 | 1·23 |
| 38. Intermediate "B", *Devon* (p. 381) | ♂ | ... | 3 | 14 | 11 | 1 | ... | 29 | 2·34 |
| | ♀ | 14 | 8 | 5 | 2 | ... | ... | 29 | 0·83 |
| *39. Ipswich, *Suffolk* (to 6/vii) . | ♂ | 1 | 19 | 135 | 44 | 9 | 2 | 210 | 2·22 |
| | ♀ | 11 | 8 | 4 | 3 | ... | ... | 26 | 0·96 |
| 40. Ipswich, *Suffolk* (from 20/vii) . | ♂ | 92 | 32 | 49 | 20 | 8 | 1 | 82 | 2·40 |
| | ♀ | 91 | 33 | 13 | 2 | 1 | ... | 140 | 0·49 |
| 41. Kelly, *Devon* . . | ♂ | 1 | 5 | 22 | 11 | 2 | 1 | 42 | 2·26 |
| | ♀ | 50 | 23 | 15 | 10 | 2 | ... | 100 | 0·91 |
| 42. Lewannick, *Cornwall* | ♂ | 2 | 7 | 79 | 29 | 9 | ... | 126 | 2·29 |
| | ♀ | 80 | 23 | 43 | 8 | 1 | ... | 155 | 0·88 |

APPENDIX 2—*continued*

*Spot-distributions of* Maniola jurtina, *1957*

| Locality | | Spots | | | | | | Total | Spot-av. |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | | |
| 43. Middleton East, *Hampshire* | ♂ | 7 | 25 | 164 | 37 | 6 | ... | 239 | 2·04 |
| | ♀ | 126 | 41 | 20 | 3 | ... | ... | 190 | 0·47 |
| 44. Middleton West, *Hampshire* | ♂ | 2 | 7 | 71 | 16 | 8 | ... | 104 | 2·20 |
| | ♀ | 48 | 16 | 5 | ... | ... | ... | 69 | 0·38 |
| 45. Newbury, *Berkshire*. | ♂ | ... | 7 | 50 | 23 | 9 | 1 | 90 | 2·41 |
| | ♀ | 25 | 6 | 17 | 1 | ... | ... | 51 | 0·88 |
| 46. North Pilistreet, *Devon* | ♂ | ... | 3 | 25 | 9 | 3 | ... | 40 | 2·30 |
| | ♀ | 26 | 11 | 8 | 4 | 1 | ... | 50 | 0·86 |
| 47. Newton Abbot, *Devon* | ♂ | 1 | 3 | 24 | 6 | ... | ... | 34 | 2·03 |
| | ♀ | 32 | 20 | 8 | 2 | ... | ... | 62 | 0·68 |
| *48. Oxford, Medley Manor (to 5/vii) | ♂ | 4 | 23 | 127 | 17 | 4 | ... | 175 | 1·97 |
| | ♀ | 25 | 17 | 21 | 5 | ... | ... | 68 | 1·09 |
| 49. Oxford, Medley Manor (24/vii) | ♂ | ... | 6 | 14 | 6 | ... | 1 | 27 | 2·11 |
| | ♀ | 16 | 9 | 2 | 1 | ... | ... | 28 | 0·57 |
| *50. Oxford, Parks (7/vii) | ♂ | 2 | 10 | 56 | 16 | 5 | ... | 89 | 2·13 |
| | ♀ | 54 | 21 | 23 | 6 | ... | ... | 104 | 0·82 |
| 51. Oxford, Parks (23, 29/vii) | ♂ | ... | 6 | 25 | 8 | 1 | ... | 40 | 2·10 |
| | ♀ | 53 | 19 | 11 | 1 | ... | 1 | 85 | 0·58 |
| 52. Pilistreet, *Devon* | ♂ | ... | ... | 25 | 12 | 2 | ... | 39 | 2·41 |
| | ♀ | 51 | 20 | 8 | 6 | ... | ... | 85 | 0·64 |
| 53. Rugby, *Warwickshire* | ♂ | 1 | 1 | 16 | 4 | ... | ... | 22 | 2·05 |
| | ♀ | 11 | 2 | 2 | ... | ... | ... | 15 | 0·40 |
| 54. Sherborne, *Dorset* | ♂ | ... | 6 | 57 | 12 | 2 | ... | 77 | 2·13 |
| | ♀ | 21 | 11 | 7 | 1 | ... | ... | 40 | 0·70 |
| 55. Taunton, *Somerset* | ♂ | 2 | 7 | 75 | 20 | 2 | ... | 106 | 2·12 |
| | ♀ | 15 | 11 | 9 | 3 | ... | ... | 38 | 1·00 |
| 56. Tamar East, *Devon* | ♂ | ... | 2 | 23 | 11 | 5 | ... | 41 | 2·46 |
| | ♀ | 67 | 36 | 34 | 5 | ... | ... | 142 | 0·84 |
| 57. Tamar West, *Cornwall* | ♂ | 2 | 4 | 35 | 9 | 4 | 2 | 56 | 2·27 |
| | ♀ | 84 | 32 | 41 | 12 | ... | ... | 169 | 0·89 |
| 58. Tiverton, *Devon* | ♂ | 1 | 3 | 37 | 10 | 3 | 1† | 55 | 2·27 |
| | ♀ | 8 | 11 | 6 | 3 | ... | ... | 28 | 1·14 |
| 59. Worthy Down, *Hampshire* | ♂ | 4 | 9 | 71 | 20 | 3 | ... | 107 | 2·08 |
| | ♀ | 73 | 27 | 11 | 3 | ... | ... | 114 | 0·51 |

* Arbitrary divisions have been made in these sets of data to illustrate the difference between the early and later parts of the emergence. Copies of these data giving complete details of dates of capture have been deposited in the Radcliffe Library, Oxford.

† This specimen had six spots.

## APPENDIX 3

*Data indicating intra-seasonal homogeneity at two locations in 1957*

| Locality | | Spots | | | | | | Total | Spot-av. |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | | |
| Middleton East, *Hampshire* (18/vi) | ♂ | 1 | 6 | 43 | 11 | ... | ... | 61 | 2·05 |
| (30/vi) . . | ♂ | 1 | 8 | 14 | 4 | ... | ... | 21 | 2·00 |
| | ♀ | 9 | 1 | ... | 1 | ... | ... | 11 | 0·36 |
| (6/vii) . . | ♂ | 5 | 14 | 84 | 22 | 6 | ... | 131 | 2·08 |
| | ♀ | 21 | 7 | 6 | ... | ... | ... | 34 | 0·56 |
| (27/vii) . . | ♀ | 42 | 13 | 3 | ... | ... | ... | 58 | 0·33 |
| (1/ix) . . | ♂ | ... | 2 | 17 | ... | ... | ... | 19 | 1·89 |
| | ♀ | 32 | 14 | 7 | 2 | ... | ... | 55 | 0·62 |
| (2/ix) . . | ♂ | ... | 1 | 6 | ... | ... | ... | 7 | ... |
| | ♀ | 22 | 6 | 4 | ... | ... | ... | 32 | 0·44 |

If the females are classed into those at 0 spots and those at one or more than one spots, and if the samples are grouped into early (30/vi and 6/vii), middle (27/vii) and late (1/ix and 2/ix) emergences, then $\chi^2_{(2)} = 1{\cdot}67$; $0{\cdot}5 > P > 0{\cdot}3$.

| Locality | | Spots | | | | | | Total | Spot-av. |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | | |
| Middleton West, *Hampshire* (18/vi) | ♂ | 1 | 5 | 33 | 6 | 5 | ... | 50 | 2·18 |
| | ♀ | 1 | 1 | ... | ... | ... | ... | 2 | ... |
| (6/vii) . . | ♂ | ... | 1 | 30 | 9 | 2 | ... | 42 | 2·29 |
| | ♀ | 12 | 5 | 2 | ... | ... | ... | 19 | 0·47 |
| (27/vii) . . | ♂ | 1 | 1 | 8 | 1 | 1 | ... | 12 | 2·00 |
| | ♀ | 15 | 6 | 2 | ... | ... | ... | 23 | 0·43 |
| (16/viii) . . | ♀ | 20 | 4 | 1 | ... | ... | ... | 25 | 0·24 |

Classifying the females as above in the case of Middleton East, $\chi^2_{(2)} = 2{\cdot}07$; $0{\cdot}5 > P > 0{\cdot}3$.

## APPENDIX 4

*Data indicating intra-seasonal shift from populations at Burham Down,* Kent, *scored by J. D. F. Frazer*

| Locality | | Spots | | | | | | Total | Spot-av. |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | | |
| Locality "A", 1955 . | ♂ | ... | 3 | 58 | 6 | 3 | ... | 70 | 2·13 |
| (to 23/vii) | ♀ | 18 | 4 | 11 | 2 | ... | ... | 35 | 0·91 |
| (after 23/vii) . | ♂ | 5 | 15 | 110 | 5 | ... | 1 | 136 | 1·88 |
| | ♀ | 208 | 36 | 19 | 2 | ... | ... | 265 | 0·30 |
| Locality "A", 1956 . | ♂ | 4 | 36 | 308 | 92 | 18 | 3 | 461 | 2·20 |
| (to 22/vii) | ♀ | 48 | 15 | 20 | 4 | ... | ... | 87 | 0·77 |
| (26/vii-2/viii) . | ♂ | ... | 6 | 69 | 13 | 3 | 1 | 92 | 2·17 |
| | ♀ | 21 | 5 | 2 | ... | ... | ... | 28 | 0·32 |
| (after 2/viii) . | ♂ | 4 | 7 | 82 | 1 | 1 | ... | 95 | 1·87 |
| | ♀ | 105 | 29 | 14 | 3 | 1 | ... | 152 | 0·46 |
| Locality "A", 1957 . | ♂ | 2 | 15 | 256 | 73 | 23 | 6 | 375 | 2·31 |
| (to 30/vi) | ♀ | 27 | 6 | 15 | 5 | ... | ... | 53 | 0·96 |
| (7 and 10/vii) . | ♂ | 1 | 8 | 83 | 20 | 11 | ... | 120 | 2·22 |
| | ♀ | 32 | 9 | 3 | 5 | ... | ... | 49 | 0·61 |

Arbitrary divisions have been made in these data to illustrate the differences between the various phases of the emergences. Each phase appears to be quite homogeneous and to be sharply distinguishable from other phases of the same emergence : all these data will be the subject of further, more detailed analysis. Both sexes appear to change simultaneously in 1955 ; in 1956 there is a short period during which the females have changed but not the males ; in 1957 there is no evidence of any change in the males up to July 10.

Copies of these data giving complete details of dates of capture and some further information concerning Locality "B" (two miles from Locality "A"), where no intra-seasonal shift could be detected in 1957, have been deposited in the Radcliffe Library, Oxford.

# DEPENDENCE OF GENOTYPIC VIABILITIES ON CO-EXISTING GENOTYPES IN *DROSOPHILA*

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## 1. INTRODUCTION

HALDANE in 1924 discussed the concepts of familial selection whereby the size of a family is severely limited by the food supply, more embryos being produced than can exist. In the case of mixed litters, in a mammal for example, there will be some genotypes somewhat weaker or less viable than the others. Haldane gives an illustrative example. He considers three litters of 20 embryos each, the first consisting of wholly strong types, the second of 10 strong and 10 weak, and the third of 20 weak. He then supposes that only 10 embryos survive and that out of equal numbers, 50 per cent. more of the strong survive. Thus the survivors are 10 strong from the first litter, 6 strong and 4 weak from the second and 10 weak from the third totalling 16 to 14. If competition had been free, the numbers would have been 18 to 12.

In *Drosophila pseudo-obscura* it has been shown (Birch, 1955) that the main competitive effect is between larvæ, with little competition between adults. Lewontin (1955), using *Drosophila melanogaster*, made a study of the larval viabilities of twenty-two strains of varying population densities. Larval viabilities varied but usually showed optimal viability at a moderate level of competition. He studied the viabilities of these strains mixed with a white-eyed stock as well, and found a change in the optimal density, some showing increased and some decreased viability compared with the pure strains. The conclusion is that the viability of a genotype is a function of the other genotypes co-existing with it; the result of any particular combination of genotypes not being predictable on the basis of the genotypes tested in isolation.

In a two-point backcross linkage experiment the proportion of the competing genotypes is different in each phase. Considering such an experiment for two factors *a* and *b* which are somewhat inviable in the recessive state, four genotypes are obtained in the offspring :

$$AB/ab,\ Ab/ab,\ aB/ab,\ ab/ab.$$

In coupling, the parentals are $AB/ab$ and $ab/ab$, the most and least viable genotypes respectively, and if competition is severe, there would be a large elimination of $ab/ab$. In the repulsion phase, the parentals are $Ab/ab$ and $aB/ab$, and assuming approximately equal inviabilities of *a* and *b* there will be no great difference in elimination between these. The recombinants $AB/ab$ and $ab/ab$, which are fewer in number will

not be in competition with each other to any great extent but rather with the parentals and as a result the proportion of *ab/ab* offspring eliminated will not be as great as in the coupling phase. Hence in such a situation, the ratio of *AB/ab* : *ab/ab* individuals will vary according to whether they are parentals or recombinants. The ratio of *Ab/ab* : *aB/ab* should not vary much as in both phases they will be eliminated approximately equally. However, if one of the recessive factors is very much more inviable than the other, both ratios may be expected to vary between coupling and repulsion. If only one factor is of poor viability, the other being entirely equivalent to the normal allele under severe competition, there would be no effect, as the viability of this factor would be equally distributed in each complementary pair. Under normal laboratory conditions of low competition these viability effects would in most situations be expected to be negligible.

Two series of two-point backcross experiments were therefore set up in *Drosophila* for three levels of competition to study the effect of competition on genotypic viabilities in coupling and repulsion. A preliminary report of one of the experiments has been published (Parsons, 1958).

## 2. METHOD

The two pairs of linked factors selected were white (*w*) and miniature (*m*) on the X-chromosome, and black (*b*) and vestigial (*vg*) on chromosome II. Both *w* and *m* under optimal conditions of abundant food show little viability effect. Vestigial, however, is somewhat inviable under the best of conditions and black is probably equivalent to wild-type. Thus the two pairs of factors selected exhibit different viability relationships and should lead to somewhat different results.

Flies were aged for 72 hours prior to mating to eliminate major age differences and then mated for 48 hours. The temperature was standardised at $25 \pm 1^{\circ}$ C. The three levels of competition were :

(1) One pair of flies mated in the normal half pint milk bottles with excess food (60-70 gm.). The medium used was composed of semolina, treacle and agar and was seeded with a few drops of yeast suspension.

(2) One pair of flies in small vials ($7\frac{1}{2} \times 2\frac{1}{2}$ cms.) with $5\frac{1}{2}$ gms. of food.

(3) Six pairs of flies in small vials ($7\frac{1}{2} \times 2\frac{1}{2}$ cms.) with $5\frac{1}{2}$ gms. of food.

For simplicity these three levels of competition will be termed low, medium, and high in the remainder of the paper.

The offspring from all matings were counted daily as they emerged.

## 3. DATA FOR WHITE (w)—MINIATURE (m)

The data for the three levels of competition are summarised in table 1. Assuming no competition between larvæ, the ratios *wm* : ++ and *w*+ : +*m* should not vary between coupling and repulsion. A simple contingency $\chi^2_1$ may therefore be done within each level to test this and these tests are presented in table 2.

The only significant result is for the high level of competition. In the low and medium levels genotypic viabilities do not vary appreciably between coupling and repulsion. Examination of table 1 shows that

*wm* flies are of much poorer viability than ++ at the high level in coupling, but in repulsion *wm* flies are, if anything, more viable than ++ flies. The ratio of *w*+ : +*m* flies is, however, approximately the same in coupling and repulsion.

TABLE 1

*Data for* w-m

| | | *wm* | *w*+ | +*m* | ++ | Total |
|---|---|---|---|---|---|---|
| (1) Low . . | C | 478 | 236 | 211 | 483 | 1408 |
| | R | 318 | 529 | 569 | 289 | 1705 |
| (2) Medium . | C | 409 | 205 | 236 | 407 | 1257 |
| | R | 190 | 407 | 424 | 200 | 1221 |
| (3) High . . | C | 506 | 288 | 300 | 659 | 1753 |
| | R | 580 | 976 | 1062 | 553 | 3171 |

C = Coupling. R = Repulsion.

The significance of the *wm* : ++ contrast may be interpreted as being due to differential competition between larvæ according to the proportions of the competing genotypes. In coupling the main competition is between *wm* and ++ so that *wm* flies are eliminated. In repulsion the general level of competition is lower as it is determined by the *w*+ to +*m* contrast and thus the rate of elimination of *wm* compared with ++ is lower than in coupling. This is the type of litter competition postulated by Haldane (1924) and discussed in the introduction.

TABLE 2

$\chi^2_1$ *tests for* wm : ++ *and* w+ : +m *within each level of competition*

| | *wm* : ++ | | *w*+ : +*m* | |
|---|---|---|---|---|
| | $\chi^2_1$ | P | $\chi^2_1$ | P |
| Low . . . | 0·71 | 0·3-0·5 | 2·53 | 0·1-0·2 |
| Medium . . | 0·16 | 0·5-0·7 | 0·62 | 0·3-0·5 |
| High . . | 13·56 | <0·001 | 0·18 | 0·5-0·7 |

It is of some interest to find out what effect, if any, competition between genotypes has on the recombination value. In table 3, estimates of recombination value are given for the coupling and repulsion data with a combined estimate using the product formula (Fisher, 1935-53) for each level of competition. Examination of this table shows that the combined estimates are all close to each other but between coupling and repulsion there is some variation for each level

of competition. Hence, if balanced data are collected the recombination fraction ought to be reasonably accurate. Bodmer (1959) in some artificially constructed examples has shown that this is so.

TABLE 3

*Recombination values between* w-m

| | Coupling | Repulsion | Combined estimate |
|---|---|---|---|
| Low . . . | 31·75±1·24 | 35·60±1·16 | 33·65±0·85 |
| Medium . . | 35·08±1·35 | 31·94±1·33 | 33·49±0·95 |
| High . . . | 33·54±1·13 | 35·73±0·85 | 34·63±0·71 |

Finally, the analysis of $\chi^2$ of these data as proposed by Bodmer and Parsons (1959) for balanced three-point linkage tests is given in table 4. For the analysis, the data are arranged in $2 \times 2$ Latin squares $\begin{matrix} AB \\ BA \end{matrix}$, where $A$ represents one complementary pair say *wm* and $++$ and $B$ the other.

TABLE 4

$\chi^2$ *analysis of* w-m *data*

| | $\chi^2$ | | | | | | d.f. |
|---|---|---|---|---|---|---|---|
| | Low | | Medium | | High | | |
| | A | M | A | M | A | M | |
| *Latin square of sums* | | | | | | | |
| Rows (parental heterozygotes) | 28·34 | 37·50 | ·52 | 1·64 | 408·35 | 460·22 | 1 |
| Columns (recombination) . | 324·45 | 359·95 | 268·71 | 292·57 | 446·04 | 489·94 | 1 |
| Diagonals (2-factor interaction) | ·17 | 5·88 | 1·76 | 2·98 | 21·85 | 2·42 | 1 |
| Total . . | 352·96 | 403·33 | 270·99 | 297·19 | 876·24 | 952·58 | 3 |
| *Latin square of differences* | | | | | | | |
| Rows (viability × parental heterozygotes) | ·31 | ·30 | ·002 | ·08 | 13·10 | 5·54 | 1 |
| Columns (viability × recombination) | 3·15 | 4·12 | ·27 | ·94 | 2·28 | 9·72 | 1 |
| Diagonals (main effect of *m*) | ·49 | ·11 | ·65 | ·73 | ·16 | ·64 | 1 |
| Total . . | 3·95 | 4·53 | ·922 | 1·75 | 15·54 | 15·90 | 3 |
| Main effect of *w* . . | ·03 | ·75 | 1·27 | 2·02 | 10·19 | 8·99 | 1 |
| Total . . | 356·94 | 408·60 | 273·18 | 300·96 | 901·97 | 977·47 | 7 |

A = Additive ; M = Multiplicative (analysis after taking logarithms).

There is a total of 7 degrees of freedom as there are 8 observed totals in a $2 \times 2$ Latin square split into complementary genotypes. The 7 degrees of freedom may be split into 3 for a Latin square of sums

(formed from the totals for each complementary pair), 3 for a Latin square of differences (formed from the differences between members of a complementary pair), and the remaining degree of freedom is for the sum of squares for the total of the Latin square of differences. All the major sources of variation can be isolated using this analysis provided that there are no major deviations from orthogonality (*i.e.* equality of the row totals of the Latin square of sums) when a weighted analysis would probably be somewhat more accurate.

The viability × parental heterozygote interaction may be interpreted as a component to detect whether viabilities vary between coupling and repulsion, and is in fact a component measuring differential competition according to the proportion of competing genotypes present. The viability × recombination interaction detects whether recombinants or non-recombinants have different viabilities and it is difficult to conceive a biological meaning for it. However, both of these components must be considered together, for, according to whether one gene is more or less viable than the other, these two interactions become interchanged due to the symmetry of the problem. Hence significance of either or both interactions is in itself evidence for a competition effect.

As in the three-point situation, the S.S. may be converted into $\chi^2$ values by dividing by the mean of the observations. Such $\chi^2$ values are tabulated in table 4.

At the low level of competition by far the largest $\chi^2_1$ is for recombination (table 4). The only other $\chi^2_1$ of any magnitude is that for parental heterozygotes which merely reflects the inequality of the row totals. For the medium level, with the exception of the recombination $\chi^2_1$, the other $\chi^2_1$'s are not significant.

However, for the high level, with the exception of the main effect of *m* and the viability × recombination $\chi^2_1$, the remainder of the $\chi^2_1$'s are large. The $\chi^2_1$ for recombination and parental heterozygotes are, as expected, very large. Then at a lower level of magnitude come the $\chi^2_1$'s for the two-factor interaction, main effect of *w*, and viability × parental heterozygote interaction. This analysis shows the predicted sources of variation and as expected demonstrates the validity of the larval competition effect.

The analyses of $\chi^2$ therefore confirm that there is a competition effect at the high level but not at the medium or low levels. Furthermore, there is a *wm* interaction at the high level which is not present at the two lower levels.

Table 4 also gives the analysis of $\chi^2$ after taking logarithms which assumes that viabilities act multiplicatively. It is possible that the multiplicative model may represent the mode of action of the viabilities more correctly than the additive model (Bodmer, 1959). At the high level both the viability × heterozygote and viability × recombination interactions are significant so that the competition effect is still present. The *wm* interaction, is, however, reduced to insignificance. At the

low level, a significant *wm* interaction and viability × recombination interaction ($P < \cdot 05$) become apparent. The latter, although not nearly as great as for the high level does indicate a slight difference between coupling and repulsion. It is, of course, difficult to say whether the additive or multiplicative model is more correct, although the latter seems intuitively more correct, and so a borderline case as this must not be regarded as real evidence.

## 4. DATA FOR BLACK (*b*)—VESTIGIAL (*vg*)

The data for the three levels of competition are summarised in table 5. On the whole the data are smaller than for *w-m* due to poor *vg* viability.

TABLE 5

*Data for* b-vg

| | | *bvg* | *+vg* | *b+* | *++* | Total |
|---|---|---|---|---|---|---|
| Low . | C | 354 | 48 | 52 | 378 | 832 |
| | R | 43 | 322 | 392 | 59 | 816 |
| Medium . | C | 314 | 45 | 49 | 402 | 810 |
| | R | 49 | 329 | 322 | 48 | 748 |
| High . | C | 490 | 69 | 102 | 836 | 1,497 |
| | R | 143 | 1,127 | 1,200 | 145 | 2,615 |

C = Coupling ; R = Repulsion.

In table 6, the contingency $\chi^2_1$'s for the two comparisons *bvg* : ++ and +*vg* : *b*+ are given. The high level shows significance for both comparisons but the low and medium levels do not show significance.

TABLE 6

$\chi^2_1$ *tests for* bvg : ++ *and* +vg : b+ *within each level of competition*

| | *bvg* : ++ | | +*vg* : *b*+ | |
|---|---|---|---|---|
| | $\chi^2_1$ | P | $\chi^2_1$ | P |
| Low . . . | 1·14 | 0·2-0·3 | 0·19 | 0·5 -0·7 |
| Medium . . | 1·28 | 0·2-0·3 | 0·14 | 0·7 -0·8 |
| High . . . . | 15·48 | $< 0 \cdot 001$ | 3·85 | 0·02-0·05 |

For the high level in coupling, the viability contrast +*vg* : *b*+ is almost as severe as *bvg* : ++, thus showing the poor viability of *vg* compared with the almost normal *b*. In repulsion, however, where the +*vg* : *b*+ contrast is dominant, *bvg* and ++ are almost equal and there is a slight deficiency of +*vg* compared with *b*+. The two sets of data thus exhibit entirely different genotypic viabilities. In

general, the situation as presented here, where one factor is of poor viability and the other of only slight inviability might well lead to significant differences between both contrasts in coupling and repulsion, which is in distinction to the *wm* data where *w* and *m* inviabilities were more equal and only the extreme *wm* : ++ contrast varied.

TABLE 7

*Recombination values between* b-vg

| | Coupling | Repulsion | Combined estimate |
|---|---|---|---|
| Low . . . | 12·02±1·13 | 12·50±1·16 | 12·26±0·81 |
| Medium . . | 11·60±1·13 | 12·97±1·23 | 12·27±0·83 |
| High . . . | 11·42±0·82 | 11·01±0·61 | 11·22±0·51 |

TABLE 8

$\chi^2$ *analysis of* b-vg *data*

| | $\chi^2$ | | | | | | d.f. |
|---|---|---|---|---|---|---|---|
| | Low | | Medium | | High | | |
| | A | M | A | M | A | M | |
| *Latin square of sums* | | | | | | | |
| Rows (parental heterozygotes) | ·16 | ·04 | 2·47 | ·30 | 303·97 | 332·48 | 1 |
| Columns (recombination) | 939·04 | 1602·51 | 887·66 | 1502·02 | 2480·94 | 4364·26 | 1 |
| Diagonals (2-factor interaction) | ·24 | ·14 | 2·97 | 1·40 | 190·04 | ·83 | 1 |
| Total . | 939·44 | 1602·69 | 893·10 | 1503·72 | 2974·95 | 4697·57 | 3 |
| *Latin square of differences* | | | | | | | |
| Rows (viability × parental heterozygotes) | 2·04 | 3·48 | 6·42 | 3·41 | 22·47 | 46·25 | 1 |
| Columns (viability × recombination) | 3·32 | ·46 | 3·91 | ·63 | 35·86 | 2·38 | 1 |
| Diagonals (main effect of *b*) | ·70 | ·29 | 5·20 | ·65 | 14·24 | ·57 | 1 |
| Total . | 6·06 | 4·23 | 15·53 | 4·69 | 72·57 | 49·20 | 3 |
| Main effect of *vg* . . | 7·89 | 11·17 | 4·53 | 2·05 | 50·13 | 64·47 | 1 |
| Total . | 953·39 | 1618·09 | 913·16 | 1510·45 | 3097·65 | 4811·24 | 7 |

A = Additive ; M = Multiplicative (analysis after taking logarithms).

As in the *wm* experiment, the estimation of recombination is not affected significantly by competition (table 7). If *vg* were the sole cause of viability effects, which is probably true at the two lower levels of competition, then such an effect would be distributed

equally in each pair of complementary genotypes and the resultant estimate of recombination would be completely unbiased.

In table 8, the analyses of $\chi^2$ of these data are given assuming both the additive and multiplicative models. Considering the additive model first, all levels give a significant $\chi^2_1$ for recombination and main effect of *vg*. The medium level gives, as well as these, significant $\chi^2_1$'s for the viability × heterozygote and viability × recombination interactions, and the main effect of black. The high level gives significance for all components. Hence a competition effect is indicated for the medium and high levels.

The multiplicative model provides an interesting contrast. At all levels the recombination effect remains and at the low level the *vg* effect. At the high level a very large *b-vg* interaction and viability × recombination interaction become insignificant. The viability × heterozygote interaction, main effect of *vg*, recombination, and parental heterozygote effect remain. At the medium level the only significant $\chi^2_1$ is for recombination. Throughout the three levels the change of model has the effect of reducing the viability × recombination $\chi^2_1$.

Hence both models give a significant competition effect at the high level but the logarithmic analysis does not give a competition effect at the medium level. The change of scale therefore alters the magnitude of some interactions but does not remove the severe competition effect found at the high level.

For the high level, the *b-vg* interaction is not significant on the multiplicative model, but is highly significant on the additive model. This shows that for these data, the multiplicative model is probably more correct than the additive model.

## 5. DISCUSSION

These data show that genotypic viabilities vary according to the proportions of the competing genotypes if competition is severe enough.

Of the two linkage tests presented, the date for the two factors *w* and *m* are somewhat more conclusive. They are separated by a recombination fraction between 30 per cent. and 35 per cent. so ensuring large numbers in the recombinant classes such that a genotypic viability effect can be found more easily. It seems that this recombination fraction may be optimal for detecting genotypic viability effects. For recombination fractions of 50 per cent., or independence, all four genotypes would be present in equal proportions so eliminating any differential effect between coupling and repulsion. In the case of small recombination fractions, large numbers need to be bred to get adequate totals in the recombinant classes.

It would be of interest to carry out further two-point or even three-point tests varying the levels of competition. A quick survey of the published linkage data in *Drosophila* did not reveal any suggestive

evidence of differential genotypic viabilities, but this is probably because linkage studies are not as a rule done under very crowded conditions.

Of secondary interest is the effect of changing the model by which the genes act from an additive to a multiplicative model. This change alters the magnitude of the gene effects and the various interactions but the main conclusions concerning competition hold irrespective of scale. However, in borderline cases, a change in model could result in different interpretations. Biologically, it seems more correct to say that two genes act multiplicatively. For example, if two genes are of viability 0·5, then on the additive scheme the double recessive would be lethal, and on the multiplicative scheme its viability would be 0·25. In the limit, therefore, the multiplicative scheme is favoured. Each case must, however, be judged on its own merits as genes react differently according to environmental factors and the genetic background.

The implications of viabilities of genotypes varying according to the competing genotypes present has not, as yet, been stressed much. In natural conditions, where competition is much more severe than in the artificial and optimal conditions created in the laboratory such effects must assume some importance. Selective advantages and disadvantages of a gene or genotype must be considered in relation to the food available and the types and proportions of the other genotypes present in the population. This is necessarily complex, but only by making the " model " complex can a true picture of the actual system be obtained.

## 6. SUMMARY

Two-point backcross experiments were done in coupling and repulsion for two pairs of factors in *D. melanogaster* at three levels of competition between larvæ.

One experiment was for white (*w*) and miniature (*m*). At the highest level of competition, the ratio of the extreme types *wm* and ++ differed in coupling and repulsion, but the ratio *w*+ : +*m* was the same in both phases.

The second experiment was for black (*b*) and vestigial (*vg*). At the highest level of competition, the ratios of both *bvg* : ++ and *b*+ : +*vg* varied according to the phase of the experiment. The difference between this and the *wm* experiment is because *vg* is rather inviable and *b* almost normal, which is a more unbalanced arrangement of viabilities than for *wm*.

The variable viability according to the phase of the experiment is shown to be due to competition with other genotypes, the proportion of the genotypes differing in coupling and repulsion. Thus the viability of a genotype is dependent on the proportion of the other genotypes co-existing with it.

The data are analysed by application of $\chi^2$ technique to the original data and after taking logarithms; thus changing from an additive to a multiplicative model. It is probable that the multiplicative model is more correct as some of the interactions are smaller than on the additive model.

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# NOTES AND COMMENTS

## SEGREGATION IN TETRAPLOID POTATOES

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Estimates of the amount of double reduction in tomatoes (Mather, 1935, 1936) and in potatoes (Cadman, 1942) have given values that are larger when the segregating plant was duplex for the locus considered, than when it was simplex. This has been reported for one locus in the tomato (*r*) and for three in the potato (*nx*, *p* and *d*). However, in the potato examples the differences, though suggestively large in one case, are not at all significant statistically. The values are given in table 1,

TABLE 1

*Indices of double reduction* ($\alpha$) *calculated from simplex and duplex tomato* (*Mather, 1936*) *and potato* (*Cadman, 1942*) *data*

| Gene locus | Simplex index | Duplex index | Difference | P |
|---|---|---|---|---|
| Tomato *r* . . | 0·106±0·040 | 0·272±0·014 | 0·166±0·042 | 0·0001 |
| Potato *nx* . . | 0·089±0·047 | 0·254±0·077 | 0·165±0·090 | 0·06-0·07 |
| *p* . . | 0·028±0·041 | 0·035±0·016 | 0·006±0·044 | 0·8-0·9 |
| *d* . . | 0·111±0·005 | 0·154±0·030 | 0·042±0·031 | 0·1-0·2 |

using the index of double reduction introduced by Fisher and Mather (1943) instead of the indices originally introduced by Mather; the latter are double the magnitude of the former.

It was at one time supposed (Mather, 1936) that a larger value for the index would be obtained from duplex data than from simplex data because of partner exchange between the chromosomes in a multivalent, but the fallacy in the argument leading to this conclusion was pointed out by Fisher and Mather (1943). Nevertheless the suggestion of a difference between the simplex and duplex indices remains and is capable of explanation in a different way, apart from misclassification and disturbed viability.

In tetraploid maize, it has been found (Catcheside, 1956) that numerically unbalanced gametes are quite frequent amongst those that function in zygote formation. Such numerically unbalanced gametes not only contribute to the class carrying only recessive genes, but do so to different extents when the gametes come from simplex, duplex and triplex heterozygotes respectively. The result is that numerical non-disjunction contributes differently to the quantities calculated above as purporting to be indices of double reduction respectively for the simplex and the duplex. In fact, if $\alpha$ is the true coefficient of double reduction and $x$ is the coefficient of numerical non-disjunction, being the proportion of gametes having one or three instead of two of the particular chromosome concerned, the simplex

index is in fact $\alpha(1-x)$ while the duplex index is $\frac{1}{4}x+\alpha(1-x)$. Thus the duplex index should exceed the simplex index by an amount equal to a quarter of the frequency of numerical non-disjunction for the locus. Similarly, the triplex index, strictly $\frac{1}{6}x+\alpha(1-x)$, would exceed the simplex index by an amount equal to one-half of the rate of numerical non-disjunction.

TABLE 2

*Segregation in tetraploid tomatoes and potatoes. Data of (1) Mather, 1936, (2) Cadman, 1942 and (3) Lunden, 1937. Note,* $y = \alpha(1-x)$

| Mating | Progeny class | Expectations | Tomato $r^{(1)}$ | Potato | | |
|---|---|---|---|---|---|---|
| | | | | $nx^{(2)}$ | $p^{(3)}$ | $d^{(3)}$ |
| Duplex backcross | Dom. | $\frac{1}{72}(10-x-4y)$ | 88 | 144 | 2588 | 668 |
| | Rec. | $\frac{1}{72}(2+x+4y)$ | 20 | 38 | 577 | 205 |
| | | | 108 | 182 | 3165 | 783 |
| Duplex self | Dom. | $1-\frac{1}{144}(2+x+4y)^2$ | 885 | 122 | 3414 | 839 |
| | Rec. | $\frac{1}{144}(2+x+4y)^2$ | 68 | 10 | 104 | 34 |
| | | | 953 | 132 | 3518 | 923 |
| Simplex backcross | Dom. | $\frac{1}{2}(2-y)$ | ... | 762 | 1122 | 494 |
| | Rec. | $\frac{1}{2}(2+y)$ | ... | 842 | 1151 | 570 |
| | | | | 1604 | 2273 | 1064 |
| Simplex self | Dom. | $1-\frac{1}{16}(2+y)^2$ | 605 | 122 | | |
| | Rec. | $\frac{1}{16}(2+y)^2$ | 221 | 41 | | |
| | | | 826 | 163 | | |
| Simplex backcross | Duplex | $\frac{1}{4}y$ | 3 | | | |
| | Simplex | $\frac{1}{2}(1-y)$ | 45 | | | |
| | Nulliplex | $\frac{1}{4}(2+y)$ | 68 | | | |
| | | | 116 | | | |

The differences between the simplex and duplex indices therefore yield estimates of the rates of non-disjunction. However, these are not efficient estimates, because they pay no regard to the unequal amounts of information about $x$ and $\alpha$ contributed by different classes of data and do not weight the data. Nevertheless they are useful as starting points in the computation of efficient estimates.

Efficient estimates of both $\alpha$ and $x$ may be obtained by joint estimation using a maximum likelihood method, which has been applied to similar data obtained for the sugary locus in maize (Catcheside, 1956). The data available in the tomato and potato and expectations for different classes of segregation are given in table 2. The estimates computed from them are given in table 3. The rates of non-disjunction for $p$ and $d$ agree very well with one another, but that for $nx$ seems to be exceptionally large. The

mean value of the rate of non-disjunction, $\bar{x}$, in the potato is $0{\cdot}0961 \pm 0{\cdot}1364$. These estimates represent very good fits to the observed data with a general absence of heterogeneity between the different classes of data. Thus the heterogeneity $\chi^2_{[2]}$ is 2·4107 in the case of *nx*; for *p*, $\chi^2_{[1]}$ is 0·9148 and for *d* $\chi^2_{[1]}$ is 3·7803.

The data for *r* in the tomato differ in that the dominant progeny of the simplex backcross had been further classified as to whether they were duplex or simplex (table 2). Though little credence can be placed in the large values of $x$ and $\alpha$ found (table 3) in the tomato, the various classes of the data are in fair agreement with one another. The heterogeneity $\chi^2_{[3]}$ is only 5·424.

The cytological data, with which these deductions should be reconciled, are as scanty as the genetic parameters are lacking in precision. There is no information available for any particular recognisable chromosome nor is it known whether there is an equal or unequal incidence of quadrivalent

TABLE 3

*Estimates of coefficients of numerical non-disjunction* ($x$) *and of double reduction* ($\alpha$) *in tetraploid tomatoes and potatoes*

| Gene | $x$ | $\alpha$ |
|---|---|---|
| Tomato *r* . . . | 0·665±0·168 | 0·316±0·236 |
| Potato *nx* . . . | 0·450±0·356 | 0·161±0·094 |
| *d* . . . | 0·033±0·267 | 0·148±0·032 |
| *p* . . . | 0·036±0·179 | 0·027±0·039 |

formation and numerical non-disjunction amongst the chromosomes. The available evidence will therefore have to be treated as though it were averaged over all chromosomes.

The occurrence of chromatid segregation, measured genetically as $\alpha$, is dependent upon the formation of multivalents, particularly quadrivalents. Its value is related to a number of genetical and cytological factors approximately by the formula $\alpha = $ c.q.p.d., where $c$ is the amount of crossing-over between the centromere and the locus, $q$ is the frequency of quadrivalent formation per set of four homologues, $p$ the frequency of adjacent (or parallel) co-orientation and disjunction of quadrivalents and $d$ is the frequency with which adjacent disjunction of the quadrivalent results in non-disjunction of the gene locus concerned. It has been thought useful to separate $p$ and $d$ in this way, rather than to combine them into one statistic, say $a$, which expresses the probability of adjacent disjunction for a given locus in a quadrivalent. The statistic $p$ can be derived relatively easily from cytological observations, but $d$ is much harder to determine owing to the rarity with which the two different arms of a chromosome may be separately distinguished in a quadrivalent. There is, of course, no independent evidence about the value of $c$ in the potato and tomato.

In the potato, the frequency of quadrivalents is rather low. The data gathered by Lamm (1945), Bains (1951) and Swaminathan (1954*a*, *b*) shows an average of about 2·1 quadrivalents per cell. If every one of the twelve sets of four chromosomes had an equal chance of forming a quadrivalent, the value of $q$ would be 0·175. The few illustrations of metaphase I

that have been published suggest that $p$ may be about 0·5. Likewise, since the centromeres are approximately median in potato chromosomes $d$ may be taken as 0·5. The maximum value of $c$ is probably 0·5. Hence the maximum value that $\alpha$ would be expected to take is about 0·022, on the assumption that all chromosomes have an equal chance of entering into quadrivalents. Cadman (1943) recorded a much larger rate of quadrivalent formation in Flourball, namely 5·24 per cell. This would give an average value of $q = 0{\cdot}437$ and a maximum value of $\alpha = 0{\cdot}055$.

The observed values of $\alpha$ (table 3) for the genes $nx$ and $p$ can be considered reconcilable with the maximum values theoretically possible in the light of cytological considerations. However, the value for the gene $d$ is much greater even than the value admitted by Cadman's Flourball data, which are generally considered to be exceptional. If the arguments and inferences are reliable, it should be concluded that the probability of quadrivalent formation falls unequally upon the twelve sets of chromosomes in the potato. It would then follow that the values of $\alpha$ for different unlinked loci would not necessarily reflect the relative amount of recombination with the centromere shown by each locus, apart from the possible complexities due to more than a single chiasma occurring between the centromere and a locus. The value of $\alpha$ might be small because quadrivalents were seldom formed or because adjacent orientation of quadrivalents occurred only rarely, altogether apart from the relative amount of recombination with the centromere.

The available cytological data have been scrutinised for evidence of non-random distribution of quadrivalent formation amongst the twelve sets of homologous chromosomes. On the whole, there appears to be no appreciable departure from randomness, the observed distributions agreeing very well with expectations based on this assumption. For example, in the case of Chippewa (Swaminathan, 1954), the 50 pollen mother cells have an average of 4·8 quadrivalents each. Cells were observed to have as few as two or as many as nine quadrivalents. The $\chi^2$ value, on the basis of equal probability (0·4) for all twelve sets of chromosomes, is only 3·9687. The assumption of various patterns of unequal distribution of probabilities generally results in worse agreement. Concentration of all quadrivalent formation into fewer than nine of the twelve sets is excluded as, also, is the occurrence of unit probability in more than two sets. As an example of the effect of unequal probabilities of quadrivalent formation, the assumption of equal probabilities of 0·8 for 4 sets and of 0·2 for 8 sets raises the value of $\chi^2$ to 12·3111, a value which is still not unreasonably large. Obviously, some patterns of unequal distribution of quadrivalent formation could be compatible with the cytological data, but there is no compelling evidence for either view.

As regards the tomato, Upcott (1935) observed an average of 3·84 quadrivalents per cell. Hence $q = 0{\cdot}32$ and, with $p$, $q$ and $c$ each a half, the maximum value of $\alpha$ would be 0·04.

An indication of the upper limit of $x$ may be derived from consideration of the frequency of numerically unbalanced sets at metaphase II of meiosis. For the potato, Lamm's (1945) data yield a value of 0·049 while Swaminathan's (1954) data give 0·026. These values agree with two of the genetical estimates, those for $d$ and $p$. For the tomato, Upcott's (1935) data yield $x$ not greater than 0·029. This is greatly different from the large

and uncertain estimate obtained by genetic means. Unfortunately none of our genetical estimations have sufficient precision for a satisfactory comparison.

## 1. SUMMARY

The values of double reduction previously made for various loci in the tomato and potato showed systematic differences according to whether the estimates were made from duplex or simplex data. The differences may be accounted for by the complicating effects of numerical non-disjunction.

*Acknowledgment.*—I am grateful to Professor K. Mather for directing my attention to these data and for his interest in their analysis.

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# REVIEW

**THE GENETICAL BASIS OF SELECTION. By I. Michael Lerner. John Wiley and Sons, New York, and Chapman and Hall, London. 1958. Pp. 298+xvi. 64s.**

This is the third book of Dr Lerner's that we have had the pleasure of reading. In the first, *Population Genetics and Animal Improvement* published nearly ten years ago (review : *Heredity*, *5* : 293-296), he dealt broadly with the concepts, techniques and problems of the biometrical study of continuous variation. The second, *Genetic Homeostasis* published in 1954 (review : *Heredity*, *9* : 273-274) was an essay on the phenomenon of inertia, or homeostasis, in population behaviour, which he sought to relate to stability in the development of individuals. Now he returns to the broader field with an account of selection and the genetical basis of response to it. As in the first book, Dr Lerner concerns himself largely with man-maintained populations of domestic species, especially chickens with which much of his own work has been done, and with the responses these populations show to artificial selection.

The first three chapters lay down the genetic foundation, dealing in turn with the notion of the mendelian population, with the principles and analysis of polygenic inheritance and with the properties of heritable variability in relation to population equilibria, optimal phenotypes, and inbreeding. The action of selection is next dealt with in four chapters, with special reference to the nature of the genetical variation and the methods of selection used. The final chapter looks at the prospects and needs of further experimental work. The text is amply illustrated by nearly a hundred graphs and diagrams. The treatment is delightfully and aptly described by the author where he says in his Preface ". . . the biological phenomenon of selection is approached without either the naturalist's disdain of or the mathematician's reverence for statistical formulation ". Indeed much of the mathematics, as well as unessential but nevertheless useful references, are confined to a series of thirty " boxes " separable from but appropriately interspersed in the text which they elaborate and underpin. This device will doubtless be welcomed by the student and the reader who is less well versed in statistical matters, and it is not out of place in a book which aims, at least in part, at an expository treatment suitable for use as a text-book albeit at an advanced level.

One might cavil at certain features of the book. For example, it is not easy to see how type III selection (in fig. 1.1) where the extreme phenotypes are preferred can be fairly described as stabilising. Its stabilising effect will be predominant only under limited circumstances and for limited ranges of time. In general its effect should be at least as much disruptive as stabilising and over near evolutionary numbers of generations it would be wholly disruptive to the distribution of phenotypes. Again one might wonder whether the author is well advised to omit standard errors, even when he is aiming only to illustrate ideas, for without them it is impossible to arrive at a truly balanced assessment for any experimental situation. One may wonder too whether it is wise to confine the designation of selection to cases where it is genetically effective, rather than using it for any

process of discrimination among individuals as prospective parents while recognising that its effectiveness and consequences will vary with the causation of the differences among these individuals.

These and their like are, however, but minor cavils. They cannot obscure the essential worth of the book, containing as it does the best account of artificial selection, its relation to and its consequences for the genetical situation yet to be produced, and with so valuable an emphasis on points such as the economics of the selection procedures described in chapter 5. If one reads it without, perhaps, the full thrill of stimulation Dr Lerner's earlier books gave us it is because the early pioneering days are over : biometrical genetics as we know it is becoming orthodox and possibly a little staid. Indeed one gets the impression from his concluding pages that this is an opinion Dr Lerner would share. But be this as it may, in one respect there has been no change. The quality of the treatment and the mastery of the presentation in this, as in the earlier books, is a pleasure and a stimulus to us all.

KENNETH MATHER.

# GENETICAL SOCIETY OF GREAT BRITAIN

## ABSTRACTS OF Papers read at the HUNDRED AND TWENTY-NINTH MEETING of the Society, held on 19th and 20th March 1959, at the UNIVERSITY OF BIRMINGHAM

### POLYPLOIDY IN WHEAT

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There are only bivalents at meiosis in the hexaploid bread wheat, *Triticum vulgare*, although its three genomes are derived from cytologically related diploid species and equivalent chromosomes of the three genomes are genetically very similar, as has been shown by nullisomic-tetrasomic compensation. Thus wheat has acquired a diploid form of meiotic behaviour in which the pairing affinity between equivalent chromosomes in different genomes either no longer occurs or is no longer expressed. The diploid behaviour is controlled by a gene, or genes, on one chromosome, in the absence of which intergenome pairing takes place and wheat ceases to behave as a classical autosyndetic allopolyploid.

Evidence of the localised genetic control of a purely bivalent forming regime makes possible a simplified concept of evolution of polyploidy, and may influence attitudes to some practical breeding problems.

### REVERSIONS OF THE LEUCINE AUXOTROPH *LEU-151* OF *SALMONELLA TYPHIMURIUM*

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" Reversions " of the leucine auxotroph *leu-151* of *Salmonella typhimurium* to leucine independence are of three types, fast, slow and unstable, distinguishable by their growth in the absence of leucine. The fast growing type is probably due to a back mutation of *leu-151* to *leu-+*. The slow type has been shown by transduction tests to be due to a suppressor mutation, (*su-leu*) at a locus which is linked to *leu-151* and to *ara-9*. This suppressor is allele specific in that it has no effect in combination with *leu-39*. A linkage map of the region has been plotted and the order of the markers shown to be *su-leu*, *leu-151*, *leu-39*, *arg-9*. The unstable type, also slow growing, spontaneously reverts to auxotrophy at a very high rate. The instability has been shown not to extend to the *methionine A* and *tryptophane B* loci, in that the mutation rates of *me A-22* and *try B-2* to prototrophy are unaffected.

### INDUCED MUTANTS IN *COPRINUS LAGOPUS*

G. E. ANDERSON

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Some 250 mutants have been isolated in *Coprinus lagopus* following ultra-violet irradiation of oidia. The mutants obtained were largely biochemical with the greatest proportion showing requirements for adenine and methionine. Those requiring amino acids, vitamins and purines (excluding adenine and methionine) were less frequent. Several methods of mutant isolation were tested. The transfer of colonies from seeded plates to complete slants before testing to minimal medium appeared to give a better result than testing colonies directly to minimal medium

and rescuing non-growers (auxotrophs) to complete medium. The total isolation method produced a yield of approximately 1 per cent. mutants among survivors whilst filtration enrichment doubled this figure. Thirty-six auxotrophic and seven morphological mutants have been tested for linkage with the mating type loci. Ten of the auxotrophs are lined with the *A* locus and include mutants with requirements for methionine, paba and arginine. Four responding to choline are linked with the *B* locus. They fall into three groups when tested on intermediates in choline synthesis. In conjunction with Dr P. R. Day tetrad analysis of two *A* and three *B* linked markers was used to determine their linear order and their position in relation to the centromere.

## THE EFFECT OF RANDOM FLUCTUATION ON GROUPS OF RAPIDLY EVOLVING POPULATIONS

J. L. CROSBY

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By use of high-speed computation techniques, an analysis has been made of the Sewall Wright effect in rapidly evolving populations, which do not seem to be amenable to direct statistical treatment. The effect is shown to be considerable even in large populations. The principle is extended to investigate the effect of random fluctuations on gene flow between populations, and its effect in determining population pattern. Primrose populations containing homostyle plants are chosen as the models for this investigation.

## SOME GENETIC EXPERIMENTS WITH STERNOPLEURAL ASYMMETRY IN *DROSOPHILA*

E. C. R. REEVE

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Left-right asymmetry of sternopleural bristles in *Drosophila* provides perhaps the most recondite character in the current repertory of quantitative inheritance. Dismissed, on the one hand, as manifestations of " developmental noise " (by analogy with information theory), variations in the level of asymmetry have also been interpreted as a " useful measure of developmental homeostasis ". Of particular interest is the fact that the level of asymmetry is at least partially under genetic control, since it was reduced when two inbred lines were crossed, and responded to selection from the $F_2$. This appears to form a striking contrast with the behaviour of the " antero-posterior asymmetry " of bristle number on the abdominal sternites, which is not easily affected genetically.

Experiments to be described indicate that two wild stocks of *D. melanogaster* both had a small but significant genetic variance for sternopleural asymmetry, and provide further data on the effects of crossing homozygous lines. In the light of these experiments some difficulties in interpreting the behaviour of the character will be examined.

## COUPLING AND REPULSION LINKAGES UNDER DISRUPTIVE AND STABILISING SELECTION

J. M. THODAY

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Two lines of *D. melanogaster* have been maintained for seventeen generations, one under disruptive selection with negative assortative mating, the other under stabilising selection. Both were initiated from the same $F_1$ cultures of a cross between a *vg*/*vg* high sternopleural chæta number line (mean 40 chætæ) and a *se cp e* stock

(mean 15 chætæ). It was known that important differences affecting chæta number occurred in the *se cp* region of chromosome III. Selection (1 in 20) began in $F_2$. Flies were selected for chæta-number: no selection for marker phenotypes was exercised.

Stabilising selection reduced variance from $F_2$ level (30) to approximately $F_1$ level (5). *vg*, *e* and *cp* were largely eliminated, and the majority of the third chromosomes in the line became *se*+.

Disruptive selection stabilised variance at a level roughly midway between that of $F_2$ and that of $F_1$. The line became homozygous *vg* and *e* but established a testcross system $++/se\ cp \times se\ cp/se\ cp$. It could be shown that + and *se* were linked with genes with strong effects on chæta number, and that recombination was continually breaking down the coupling linkage of these genes, and the linkage of some of them at least to the *se* locus.

The experiment therefore demonstrates that disruptive selection can maintain coupling linkages of relevant genes against considerable "recombination pressure" and that stabilising selection can promote repulsion linkages.

The disruptive selection line, at the end, was polymorphic for the loci *se* and *cp*, and gave a strongly bimodal distribution for chæta number.

## GENE-ENVIRONMENT INTERACTION IN THE GROWTH OF *DROSOPHILA*

F. W. ROBERTSON

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The problem of genetic differences in ability to withstand a deficient diet during larval growth in *Drosophila* has been studied by rearing flies on chemically defined aseptic media in which the general nutrient concentration is varied or the amino-acid supply is restricted. Performance, measured by the relative decline in adult body size compared with growth under the most favourable conditions, may be regarded as an important constituent of fitness. Tests have been carried out on inbred lines, strains selected for large and small body size and on various crosses between them. They suggest a fairly systematic tendency for departure from the original mean in either direction to be associated with progressive loss of resistance to sparse diets and this can be detected after only a few generations of mass selection. Since the environmental conditions are repeatable, it should be possible to place these relations on a more systematic, quantitative basis.

## SOME GENETIC IMPLICATIONS OF MATERNAL EFFECTS

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In a crossbreeding experiment involving all types of matings among Friesian, Ayrshire, and Jersey cattle, the effect of maternal size has been investigated, based on various comparisons of reciprocal crosses, using data on weight and body size from birth to two years of age. Among the thirteen characters analysed, there was a close relationship at birth between the relative maturity of the characters and the relative extent of their maternal effects. The type of maternal effect depended on whether the crossbred calf is from the larger or smaller maternal breed, but in all cases the effect diminished during the calf's growth. The information will be interpreted in terms of variation in expression of the genotype for body size from birth to maturity. The main conclusions will indicate how an understanding of maternal effects might facilitate a selection programme by permitting selection to be carried out at an early age.

## MATERNAL AND SEX-LINKED EFFECTS ON GROWTH AND FORM IN THE FOWL

A. G. COCK

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C and I are inbred lines of White Leghorn (F~99 per cent.). C♀♀ weigh approximately 1600 g. at sexual maturity and I♀♀ 1300 g. The $F_1$ I♂×C♀ is heavier in both sexes at all ages than the reciprocal cross; average weights of ♀♀ at sexual maturity are: I×C—1670 g. (171 days); C×I—1540 g. (169 days). In ♂♂ (homogametic sex) the difference between reciprocals is smaller, and crosses of $F_1$ ♀♀ with ♂♂ of a third inbred line support the view that about one-third of the difference between reciprocal $F_1$ ♀♀ is due to sex-linkage, the smaller (I) line having the "larger" X-chromosome.

Despite their lower body weight, C×I ♀♀ are identical with I×C ♀♀ in mean length of tarsometatarsus and slightly exceed them in thickness of shaft of tarsometatarsus (measurements on dried bones of ♀♀ killed at 490 days of age). Skeletal dimensions, as well as body weight, are subject to hatch effects, but may change in opposite directions from one hatch to another. Thus differences between hatches as well as between reciprocal $F_1$'s reveal changes in conformation as well as size. The interpretation of these changes in terms of relative growth-rates will be discussed.

## SPONTANEOUS MUTATIONS IN DRY *NEUROSPORA* CONIDIA

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Atwood's heterocaryon for the scoring of recessive lethals was used in an attack on the problem whether spontaneous mutations arise in non-duplicating genes. This method, in contrast to previous ones, detects only mutations which arise before plating, and excludes those which arise on the plate through replication errors. The results show that lethals arise at a constant rate in dry conidia. Under the conditions used, this rate was 0·3 per cent. per week at 32°, and considerably lower at 4°. When spores kept at 32° were transferred to growth medium, a high proportion of the already present lethals was lost during the initial establishment of a growing front; subsequently, mutation frequency increased at about twice the rate found for dry conidia. A spore sample which had spent the first six months at 4° and the seventh at 32° contained at least as many lethals as spore samples which had been kept at 32° throughout. If this result can be confirmed, it suggests the existence of a two- (or multiple-) step process, with only the final step showing marked temperature dependance.

## POLYGENIC MUTATIONS AFFECTING VIABILITY

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Data on the viability of irradiated X-chromosomes of *Drosophila melanogaster*, summarised in a paper by Lüning and Jonsson (1957), have been analysed in an attempt to determine the characteristics of "normal" treated chromosomes, that is, after the exclusion of lethals and semi-lethals. The most definite effect is a reduction of mean viability of males with treated chromosomes relative to the controls. This is highly significant statistically. At first sight all chromosomes appear to be affected uniformly: there is no suggestion of bimodality, not even of increased variance. After applying some corrections, however, there appears to be a slight increase in variance of treated chromosomes. As far as it is possible to judge from the published

material, the data appear to be very similar to those earlier obtained by Timoféef-Ressovsky (1935) and Kerkis (1938). It is the interpretation which is different: that the results are due to very small mutations of very high incidence, such that after 2000 *r* to mature sperm, the incidence of mutations is greater than one per chromosome.

## A MODEL FOR RECOMBINATION BEFORE ZYGOTENE PAIRING

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Recombination by copy-choice requires homologous contact between chromosomes at the time of replication. In apparent contradiction to this, replication of DNA occurs before zygotene pairing in a number of organisms.

In *Aspergillus* and other organisms, only a small fraction of the total genetic length is available for recombination to take place in any one cell. The necessary condition for recombination is called effective pairing to distinguish it from cytologically observable pairing. In *Aspergillus*, effectively paired segments have a mean length of about 0·4 map units and the mean exchange frequency per segment is 0·6. This corresponds to a recombination fraction of 35 per cent. and means that the frequency of recombination between loosely linked loci is determined principally by the frequency of effective pairing between them; effective pairing is a limiting factor in recombination.

If effective pairing were equivalent to homologous contact, chromosomes need be in contact at only a few points at the time of recombination to account for their genetic length. There need be no cytologically observable pairing. There is no direct evidence that effective pairing is equivalent to homologous contact, but if it were, the well-known observation that chiasma frequencies are greater in triploids and trisomics than in the corresponding diploid might be accounted for in terms of increased probability of homologous contact between pairs of chromosomes when three are present as compared with two.

## ALTERNATIVES TO HETEROSIS

J. H. EDWARDS

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The diversity of the blood groups in man and other vertebrates lies within Ford's definition of polymorphism, and heterosis is a sufficient mechanism for the development and maintenance of this variability. Although it is sufficient, it is not necessary and in view of the absence of any unequivocal evidence of any specific heterotic advantage in any serological character various other explanations will be considered.

Opportunities for the expression of differential fitness of various genes may occur through selective mating, selective gamete conjugation, maternal-fœtal interactions, and differential morbidity after birth. Maternal-fœtal interactions, while undoubtedly complicating the situation, cannot explain it as the evolution of serological individuality preceded placentation.

In particular it will be maintained that:

(1) Inbreeding depression associated with a variable amount of inbreeding will lead to the perpetuation of many forms of genetic variability, not all of which need contribute to increased vigour on outbreeding.

(2) A tendency for gametes with dissimilar surface properties to conjugate preferentially will lead to an extremely stable variability if these properties are determined by the gametic genotype. This selection may or may not be of any biological significance in the viability of the diploid organism.

## A MUTABLE GENE IN *DELPHINIUM AJACIS*

G. W. P. DAWSON

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$p$ (pink), $p^b$ (blue), $p^l$ (lavender) and $p^*$ are alleles at a locus controlling flower colour. $p^*$ mutates at a high rate to $p^b$ and to an allele which has a low rate and complicated pattern of mutation to $p^b$. The rate of mutation of two doses of $p^*$ to $p^b$ is twice the rate of mutation of one dose of $p^*$, in both the sepals and the reproductive tissues. $p^*$ has a high mutation rate only when a dominant activator gene is present.

The inheritance of mottled pink/rose flowers and mosaic green/yellow leaves will also be discussed briefly.

## DIFFERENTIATION IN THE POTATO TUBER

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*Plant Breeding Institute, Cambridge*

Tubers of the cultivated tetraploid potato (*Solanum tuberosum* L.) may have anthocyanin pigments in the cells of either the periderm or the outer cortex. The results described here are for pigmentation in the periderm, which in potato tubers is formed nearly entirely from the epidermis.

When eyes were removed from the white-splashed-purple tubers of a seedling so that adventitious buds were produced from the inner tissues, some of the resulting plants had white tubers. Clonal generations from the original white-splashed-purple tubers and from the white tubers obtained by the eye-excision experiment have remained true to their respective types.

Hybrid progenies from crosses of white tubered varieties on to the plants with white-splashed-purple tubers and on to the plants with white tubers both showed similar segregations of approximately 11 white-splashed-colour : 13 white.

The simplest explanation of these results would appear to be that the cells of the inner tissues of a tuber differ from those of the epidermis (and the periderm which it produces) in not being able to produce the purple pigment, and that they do not regain this power even when they are made to become epidermal cells and even although their nuclei contain the gene for white-splashed-colour periderm.

## Corrigendum

ENNER, *Heredity*, Vol. 13, *p.* 284, *line* 10-11 "reciprocal crosses of *Cr pictirubata*" should be "*cr pictirubata*".